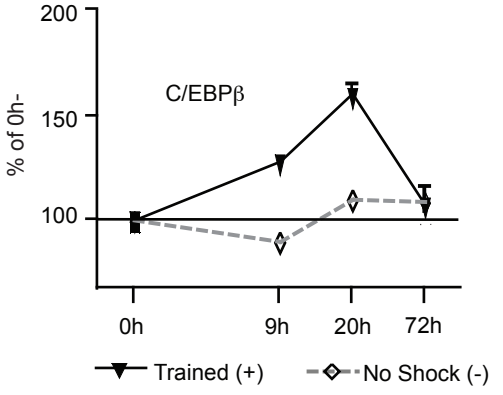
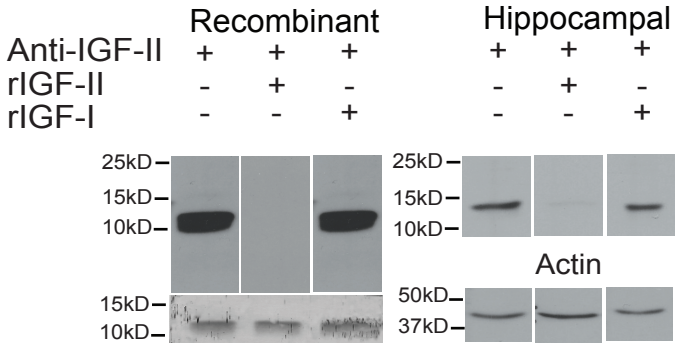
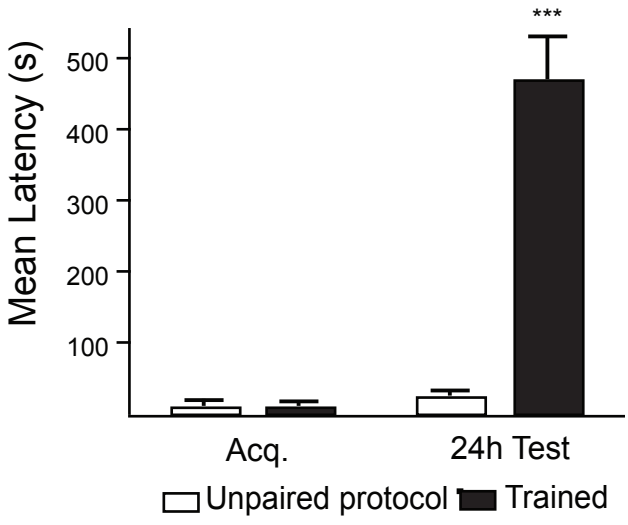


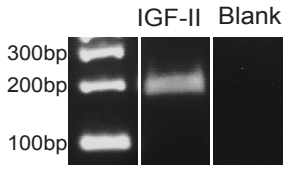

**Supplementary Figure S1: Hippocampal C/EBPβ increases after IA training**
Taubenfeld et al.(ref. 20) showed that, compare to rats that were exposed to the IA box without footshock (No Shock), hippocampal expression of C/EBPβ significantly increased between 6-9h after training and returns to baseline by 48h after training.



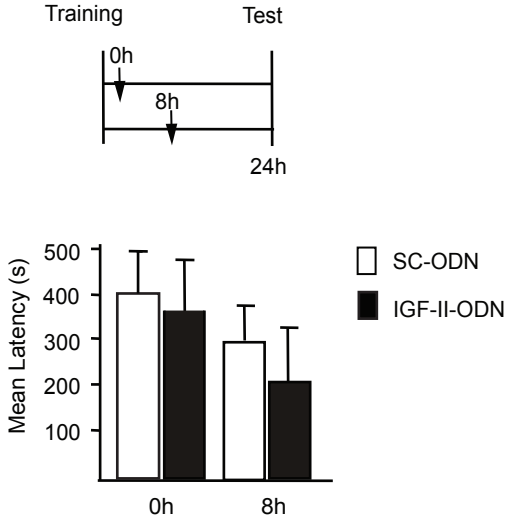

**Supplementary Figure S2: Anti-IGF-II antibody specifically recognizes IGF-II but not IGF-I.** Western blot analyses with anti-IGF-II antibody of recombinant IGF-II and hippocampal extracts non-competed or competed with either recombinant IGF-II or IGF-I. Colloidal gold stained membranes and actin were used as loading controls.




**Supplementary Figure S3: Unpaired protocol does not lead to long-term memory retention.** Unpaired protocol, which dissociates context and footshock by one hour does not elicit long-term IA memory retention. Data are expressed as mean latency ± s.e.m. Two-way ANOVA comparing behavioral protocols and latency revealed a significant interaction $F(1,20)=59.84$, $P<0.0001$, behavioral protocol $F(1,20)=59.74$, $P<0.0001$ and latency $F(1,20)=67.84$, $P<0.0001$. Bonferroni post hoc test confirmed that unpaired protocol does not elicit long-term memory retention, where as training leads to significantly memory latency 24h later (***P<0.001).

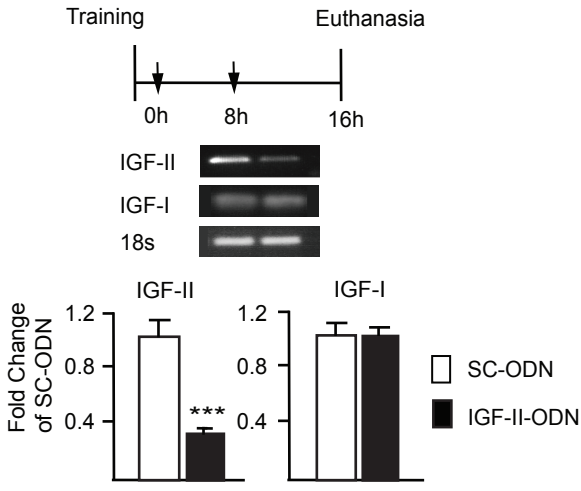



**Supplementary Figure S4: C/EBPβ binds to IGF-II promoter region of exon 1.**
PCR amplication fragment following C/EBPβ ChIP assay with primers designed to flank the IGF-II promoter region upstream of exon 1 (bp17939-18121 of sequence X17012.1) which contains a putative C/EBPβ consensus sequence.
Blank corresponds to PCR reaction carried out without the immunoprecipitated DNA. The sequence of the fragment was confirmed by DNA sequencing.



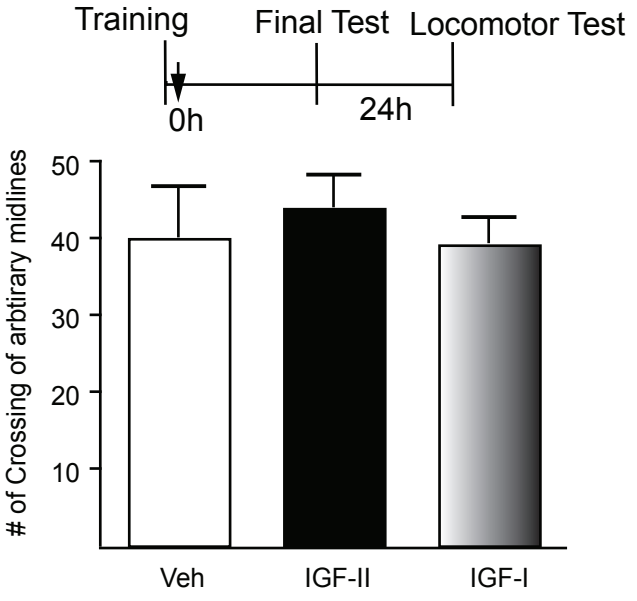

**Supplementary Figure S5: Hippocampal single injections of IGF-II-ODN do not impair long-term memory**

Injections of IGF-II-ODN either immediately after (0h) or 8h after training do not impair memory retention tested at 24h after training. Data are expressed as mean latency ± s.e.m.
0h: SC-ODN (408.2±77.9s); IGF-II-ODN (381.7±103.4s). 8h: SC-ODN (294.9±108.3s); IGF-II-ODN (208.9±118.6s) (n=8/group).

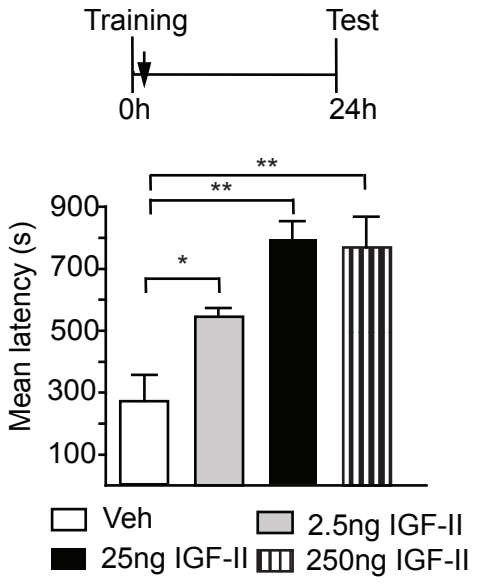



**Supplementary Figure S6: Double injections of IGF-II-ODN selectively knock down IGF-II, but not IGF-I mRNA.**

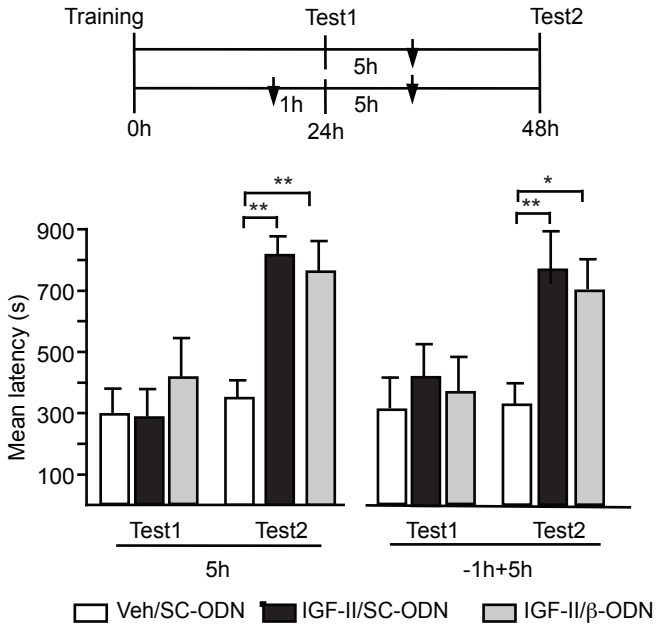
Representative example and real-time quantitative PCR of IGF-II and IGF-II show that double Injections of IGF-II-ODN into the hippocampus immediately after (0h) and 8h after training knock down endogenous levels of IGF-II, but not IGF-I mRNA measured at 16h after training. Data are expressed as mean Fold change of SC-ODN± s.e.m (Student's *t*-test ***$P$=0.0006). IGF-II: (SC-ODN: 1.0±0.1; IGF-II-ODN: 0.3±0.04). IGF-I: (SC-ODN: 1.0±0.09; IGF-II-ODN:0.98±0.07). n=4/group.




**Supplementary Figure S7: IGF-II administration does not affect spontaneous locomotor activity.**
Twenty-four h after the final retention test (experiments described in Figs 3a and 4a), rats injected with either vehicle, IGF-II or IGF-I were placed in a novel box and their activity, which was measured by the number of crossings of arbitrary lines that divide the box into quadrants, in 540 sec.
Veh (n=8): 40.8± 7.0; IGF-II (n=8): 44.6± 4.1; IGF-I (n=8): 39.6± 2.3.
Data are expressed as mean number of crossing ± s.e.m.

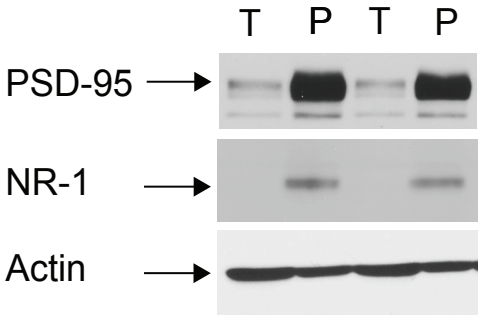



**Supplementary Figure S8: IGF-II enhances memory retention in a dose-dependent manner.** Groups of rats were injected with different doses of IGF-II immediately after training. Memory was tested 24h after training. Veh (n=4): 275.3± 83.5s; 2.5ng (n=5): 552.4± 23.3s; 25ng (n=6) 798.4±57.55s; 250ng (n=6): 758.9± 93.6s. One-way ANOVA $F(3,20)=9.37$, $P=0.0007$ followed by Newman-Keuls post-hoc test *$P<0.05$, **$P<0.01$). Data are expressed as mean latency ± s.e.m.

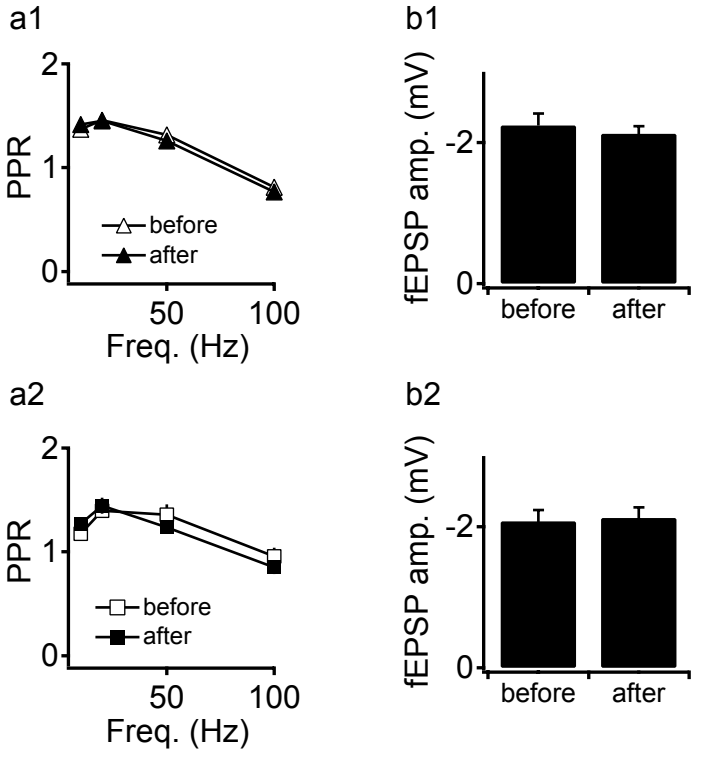



**Supplementary Figure S9: IGF-II-mediated memory enhancement does not require the function of C/EBP**β.

Neither single Injections of C/EBPβ–ODN 5h after memory retreival (Test 1), nor double injections of C/EBPβ–ODN, 1h before and 5h after Test 1, affected the IGF-II mediated memory enhancement. Data are expressed as mean latency ± s.e.m. 5h (Veh/SC-ODN: Test 1: 300.65±75.69s, Test 2: 323.6±74.1s; IGF-II/SC-ODN: Test 1: 289.2±85.3s, Test 2: 821.6±53.9s;IGF-II/β–ODN: Test 1: 419.7±121.3s, Test 2: 768.2 ±97.5s). -1h+5h (Veh/2xSC-ODN: Test 1: 315.5±96.4s, Test 2: 329.7±74.2s; IGF-II/2xSC-ODN: Test 1: 427.3±99.3s, Test 2: 743.7±137.7s; IGF-II/2xβ–ODN: Test 1: 371.8±107.5s, Test 2: 708.2 ±90.3s). n=4-6/group. (5h: two-way ANOVA $F(2,26)=2.8$, $P=0.079$ for interaction, $F(2,26)=5.93$, $P=0.0075$ for treatment, $F(1,26)=11.9$, P=0.0019 for test, Bonferroni post-hoc $^{**}P<0.01$; -1h+5h: two-way ANOVA $F(2,30)=2.53$, $P=0.0967$ for interaction, $F(2,30)=3.72$, $P=0.0361$ for treatment, $F(1,30)=10.27$, $P=0.0032$ for test, Bonferroni post-hoc $^{*}P<0.05$,$^{**}P<0.01$)

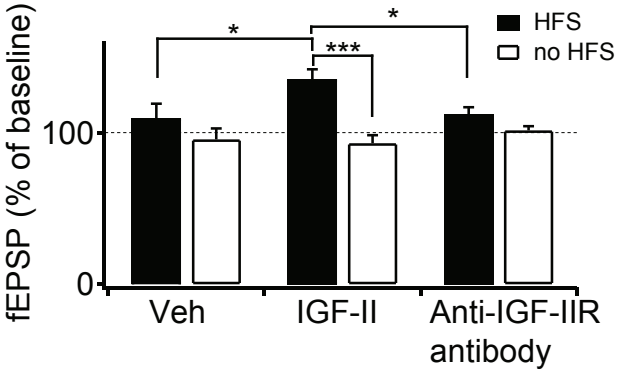



**Supplementary Figure S10: Biochemical characterization of the synaptoneurosomal fraction.**
Western blot analyses showed that synaptoneurosomal fraction (P) is enriched for
PSD95 and NMDA receptor subunit NR1 compared to total lysate (T), whereas actin is not.




**Supplementary Figure S11: a, Paired pulse ratio (PPR) and b, spike threshold are not affected by IGF-II or anti-IGF-IIR antibody.**

a1, PPRs (n=4) were obtained by evoking two fEPSPs at 10, 20, 50 and 100 Hz, before the addition of IGF-II (1.34± 0.04, 1.43± 0.02, 1.28± 0.03 and 0.78± 0.04 respectively) and in the presence of IGF-II (1.39± 0.02, 1.47± 0.04, 1.34± 0.02 and 0.84± 0.07 respectively). a2, PPRs (n=5) were obtained as a1, but either before the introduction of anti-IGF-2-R antibody (1.17± 0.06, 1.39± 0.06, 1.35± 0.09, 0.96± 0.08 at 10, 20, 50 and 100 Hz respectively) or in the presence of the antibodies (1.27± 0.04, 1.44± 0.08, 1.23± 0.05 and 0.85± 0.06 at 10, 20, 50 and 100 Hz respectively). In all cases, PPR was unaffected by drug treatment. b, Spike threshold was measured by stimulating input fibers at increasing intensities before and after treatment. Neither IGF-II (b1) (n=7, before: 2.24± 0.16; after 2.12± 0.11 mV) nor anti-IGF-IIR antibody (b2) (n=5 before 2.07± 0.16; after 2.12± 0.15 mV) produced any changes in spike threshold.




**Supplementary Figure S12: IGF-II promotes the induction of IGF-IIR dependent stable LTP.**
Average of the last 10 minutes of data plotted in Fig 6d showing that IGF-II promotes LTP maintenance after a weak LTP induction and anti-IGF-IIR antibody blocks this effect.
Depicted group differences are significant at $P<0.001$ (***) or $P<0.05$ (*); no other group differences were statistically significant.

**Table 1: Fold change of IGF-II mRNA and protein levels and IGF-I mRNA levels**

| **Figure 1a** | | **Control (h-)** | **Trained (+)** |
|---|---|---|---|
| 0h | 9 | 100 ± 11.7% | |
| 6h | 6 | 100 ± 38.3% | 90.2 ± 10.2% |
| 9h | 6 | 98.6 ± 20.8% | 102.2 ± 59.5% |
| 20h | 9-10 | 102.9 ± 16.7% | 255.5 ± 72.6% |
| 36h | 4 | 83.3 ± 25.3% | 194.7 ± 61.0% |
| **Figure 1b** | | **Fold Change of 20h-/0h-** | |
| | | IGF-II | IGF-I |
| 20h-/0h- | 6 | 1.00 ± 0.05 | 1.00 ± 0.11 |
| 20h+/0h- | 6 | 3.04 ± 0.14 | 1.05 ± 0.16 |
| **Figure 1c** | | **% of 0h-** | |
| 0h- | 8 | 100.0 ± 7.9% | |
| 20h- | 12 | 89.3 ± 18.8% | |
| 20h+ | 12 | 166.13 ± 20.6% | |
| 72h- | 8 | 99.7 ± 24.5% | |
| 72h+ | 8 | 90.0 ± 27.6% | |
| 96h+ | 4 | 92.25 ± 13.8% | |
| 96h- | 4 | 98.8 ± 14.8% | |
| **Figure 1d** | | **% of SC-ODN Unp** | |
| SC-ODN Unp | 4 | 100.0 ± 14.2% | |
| SC-ODN Trained | 8 | 223.5 ± 30.8% | |
| β-ODN Trained | 7 | 135.2 ± 17.2% | |
| β-ODN Unp | 4 | 124.9 ± 7.4% | |

**Table 2: Mean latencies of rats after training and treatment**

| **Figure 2a** | **n** | **Mean Latency (s)** | | |
|---|---|---|---|---|
| | | 0h/8h | 24h/32h | 96h/104h |
| SC-ODN | 8 | 322.7 ± 84.6 | 316.6 ± 67.5 | 256.3 ± 74.5 |
| IGF-II ODN | 8 | 96.1 ± 32.8 | 113.8 ± 55.6 | 217.7 ± 33.8 |
| Retrain | | | 413.2 ± 53.2 | |
| **Figure 2b** | | **Mean Latency (s)** | | |
| SC-ODN/IGF-I | 9 | 371.4 ± 47.0 | | |
| SC-ODN/IGF-II | 8 | 430.3 ± 56.8 | | |
| IGFII-ODN/IGF-I | 10 | 161.9 ± 33.7 | | |
| IGFII-ODN/IGF-I | 8 | 410.0 ± 46.0 | | |

**Table 3: Mean latencies or percent freezing of rats after training and treatment**

| **Figure 3a** | **n** | **Mean Latency (s)** | |
|---|---|---|---|
| | | **Test1** | **Test2** |
| Veh | 7 | 280.5 ± 69.1 | 393.32 ± 69.13 |
| IGF-II | 8 | 610.7 ± 95.9 | 818.13 ± 81.87 |
| IGF-I | 7 | 134.7 ± 71.9 | 200.68 ± 53.19 |
| **Figure 3b** | | **Acq.** | **Test** |
| Veh | 6 | 19.6 ± 6.8 | 111.4 ± 36.2 |
| IGF-II | 5 | 19.5 ± 8.1 | 455.8 ± 136.5 |

| **Figure 3c** | | **Percent freezing** | | |
|---|---|---|---|---|
| | | **Baseline** | **Context Test** | **Tone Test** |
| Veh | 7 | 3.9 ± 1.2 | 30.2 ± 5.9 | 79.5 ± 4.4 |
| IGF-II | 7 | 2.7 ± 1.0 | 49.2 ± 5.8 | 81.0 ± 4.3 |

| **Figure 3d** | | **Mean Latency (s)** |
|---|---|---|
| | | **Test** |
| Veh | 6 | 311.9 ± 104.5 |
| IGF-II | 7 | 356.4 ± 79.3 |

**Table 4: Mean latencies of rats after training and treatment**

| **Figure 4a** | **n** | **Mean Latency (s)** | |
|---|---|---|---|
| | | **Test 1** | **Final Test** |
| IGF-I | 6 | 188.2 ± 41.6 | 288.9 ± 48.7 |
| IGF-II | 9 | 245.9 ± 57.7 | 710.7 ± 100.9 |
| IGF-I NoR | 5 | - | 226.7 ± 77.3 |
| IGF-II NoR | 5 | - | 175.1 ± 39.3 |
| **Figure 4b** | | **Test 1** | **Final Test** |
| Veh | 9 | 136.0 ± 48.8 | 363.9 ± 123.8 |
| IGF-II | 9 | 164.6 ± 62.5 | 382.7 ± 133.2 |

**Table 5: Mean latencies of rats after training, reactivation and treatment**

| **Figure 5a** | **n** | **Mean Latency (s)** | |
|---|---|---|---|
| Veh | 9 | 333.7 ± 87.9 | |
| IGF-II | 6 | 777.58 ± 92.06 | |
| IGF-II/Anti-IGF-II-R | 8 | 225.9 ± 86.9s | |
| IGF-II/JB1 | 8 | 625.1 ± 121.3 | |
| Anti-IGF2-R | 8 | 460.8 ± 136.9s | |
| JB1 | 8 | 241.6 ± 91.2 | |
| **Figure 5b** | | **Mean Latency (s)** | |
| IgG | 6 | 341.4 ± 73.2 | |
| Anti-IGF-IIR antibody | 6 | 62.6 ± 17.4 | |
| **Figure 5c** | | **Mean Latency (s)** | |
| | | **Test 1** | **Test 2** |
| Veh | 5 | 240.1 ± 84.7 | 283.6 ± 98.9 |
| IGF-II | 7 | 228.3 ± 59.5 | 733.8 ± 93.4 |
| IGF-II+Aniso | 8 | 241.1 ± 65.9 | 376.1 ± 65.9 |
| **Figure 5d** | | **Mean Latency (s)** | |
| Veh/SC-ODN | 5 | 282.3 ± 55.0 | |
| IGF-II/SC-ODN | 5 | 853.2 ± 31.0 | |
| IGF-II/Arc-ODN | 6 | 336.1 ± 126.7 | |
| Veh/Arc-ODN | 6 | 224.3 ± 57.5 | |

**Table 6: Fold change of western blot analyses and mean latencies of rats after training, reactivation and treatment**

| **Figure 6a** | **n** | **pCREB** | **C/EBPβ** |
|---|---|---|---|
| Naïve-Veh | 6-8 | 100.0 ± 15.2% | 100.0 ± 6.4% |
| Trained-Veh | 6-8 | 168.6 ± 20.1% | 140.9 ± 10.6% |
| Trained-IGF-II | 7 | 185.0 ± 30.0% | 161.5 ± 20.6% |
| **Figure 6b** | | **GluR1** | **GluR2** |
| Naïve-Veh | 8 | 100.0 ± 21.2% | 100.0 ± 12.0% |
| Trained-Veh | 5 | 128.9 ± 30.6% | 72.3 ± 4.9% |
| Trained-IGF-II | 5 | 261.4 ± 52.2% | 83.3 ± 10.8% |
| Trained-IGF-II + anti-IGF-IIR | 5 | 140.4 ± 37.5% | 81.4 ± 19.4% |
| **Figure 6c** | | **pGSK3β** | **GSK3β** |
| Naïve-Veh | 5 | 100.0 ± 13.2% | 100.0 ± 11.1% |
| Trained-Veh | 5 | 88.1 ± 3.4% | 99.3 ± 10.9% |
| Trained-IGF-II | 5 | 53.9 ± 5.7% | 110.4 ± 16.1% |
| Trained-IGF-II + anti-IGF-IIR | 5 | 80.3 ± 9.6% | 105.7 ± 6.9% |
| **Figure 6d** | | **Mean Latency (s)** | |
| | | **Test 1** | **Test 2** |
| Veh | 8 | 223.0 ± 51.5 | 298.4 ± 66.7 |
| SB216763 | 8 | 225.9 ± 41.5 | 332.1 ± 67.6 |
| IGF-II | 8 | 251.9 ± 63.4 | 661.61 ± 111.3 |
| IGF-II+SB216763 | 8 | 248.1 ± 49.6 | 207.5 ± 31.3 |