water. Animals were divided into two groups: control animals (n=6) received Normal Rodent Diet (NRD) (Aashirwad industries, Mohali, India) and HFD group received diet containing 58% energy from fat (lard) using standard composition [8] described (Table 1). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Punjab University, Chandigarh. Experiments were performed in accordance with Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and Indian National Academy of Science (INSA) guidelines.

| **Ingredients** | **Diet (g/kg)** |
|---|---|
| Powdered NRD | 365 |
| Lard | 310 |
| Casein | 250 |
| Cholesterol | 10 |
| Vitamin & mineral mix | 60 |
| DL-Methionine | 03 |
| Yeast powder | 01 |
| Sodium chloride | 01 |

AChE is a prominent marker in cognition loss because of its ability to breakdown the important neurotransmitter acetylcholine. Briefly, the samples were homogenized in 10 mM sodium phosphate buffer, pH 7.4 and then centrifuge at 10,000 g for 20 minute, 4°C. Supernatant was collected and used for AChE estimation. In 3.0 mL of sodium phosphate buffer (0.1 M, pH 8.0), 0.1 mL of Ellman's reagent was mixed and 0.1 mL of acetyl thiocholine iodide was added, afterwards 0.05 mL samples were added and change in absorbance over a period of 2 minute at 412 nm. Results were calculated using the molar extinction coefficient of chromophore ($1.36\times104\ M^{-1}\ cm^{-1}$). Results were expressed as µM substrate hydrolyzed/ min/mg protein.

## Statistical analysis

Data were analysed with Graphpad 6.0 software (Graphpad software Inc., CA, USA). All the values were presented as mean ± S.E.M. A two way Analysis of Variance (ANOVA) with Bonferonni post-test were used for analysis of body weight data and serum MN concentration at different time intervals. Unpaired t-test was used for analysis of central and peripheral MN concentration, average weight gain, average MN change, behavioural tests, MAO-A and MAO–B and AChE activity. Pearson correlation was used for correlation statistics. A minimum criterion for significance was set to $P \leq 0.05$.

## Results

Administration of HFD significantly increased the body weight of rats (Figure 1A) Behavioural analysis (Figure 1 B–D) showed significant increase in immobility time for HFD fed rats as compared to NRD fed rats in forced swim test (P<0.0001). Likewise transfer latency in elevated plus maze test was significantly increased (P<0.0001) in HFD fed rats. On the contrary, there was a significant decrease (P=0.0041) in total locomotor activity of HFD fed rats as compared to control (Figure 1B–D). There was significant correlation between weight gain and behavioural changes (Figure 1E–G).

Serum MN levels were decreased with weight gain (although non-significant) [F(3,52)=0.7674, P=0.5175]. MN levels in vWAT of HFD rats was significantly high (P=0.0302) as compared to control rats whereas there was no change MN concentration in BAT. On the contrary, brain MN levels were significantly decreased (P=0.0404) in HFD fed rats (Figure 2A). A significant increase (P<0.0001) in average weight gain and decrease in average change in MN concentration were observed in HFD fed rats (Figure 2B). In HFD fed animals, a significant negative correlation was found between weight gain of animals and change in their MN levels (Figure 2C) (r=-0.4098, P=0.0123). Central MAO-A and MAO-B activity was significantly enhanced in obese rats as compared to normal rats (P<0.0001) (Figure 2D). In contrast, peripheral MAO-A (in BAT and vWAT) was significantly reduced (P=0.008 and 0.0034 respectively). MAO-B activity in BAT was not changed significantly in HFD rats as compared to normal rats (P=0.0902), whereas, it was decreased significantly in vWAT of HFD fed rats as compared to control (Figure 2D). The enzymatic activity of AChE (Figure 2E) was found to be significantly increased in obese rats (P=0.0164) as compared to normal rats.

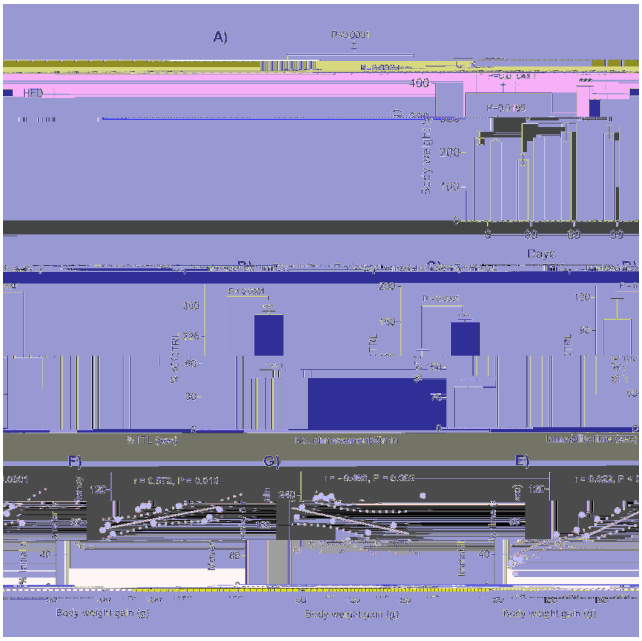


**Figure 1:** Effect of high fat diet on different behaviour paradigms in rats: [A] body weights for 90 days; [B] immobility time in forced swim test; [C] transfer latency in elevated plus maze test; [D] locomotor activity in photoactomreter; [E–G] correlation between body weight gain and different behaviour parameters (dotted line represent 95% confidence interval band). All the values (A–D) are expressed as mean ± S.E.M. (Two way ANOVA or student t-test was applied; *P<0.05 versus its respective control, **P<0.01 versus its respective control, ***P<0.001 versus its respective control, †P<0.01 versus day 0 control, ‡P<0.001 versus day 0 control). CTRL=Control (n=6), HFD=High Fat Diet (n=10), EPM=Elevated Plus Maze, ITL=Initial Transfer Latency.

## Discussion

The present study suggests a positive correlation between body weight gain and development of neuropsychiatric illnesses like depression and cognitive deficits. Further, while evaluating (a) serum and tissue (brain and vWAT/BAT) metanephrine levels (b) MAO A & B enzyme activity in brain and adipose tissue, we have established a link between perturbations in monoamine metabolism and

accumulation and abdominal obesity [15]. It underscores the importance of understanding mental health in obese patients and also suggests obesity is associated with an increased risk of developing psychiatric disorders.

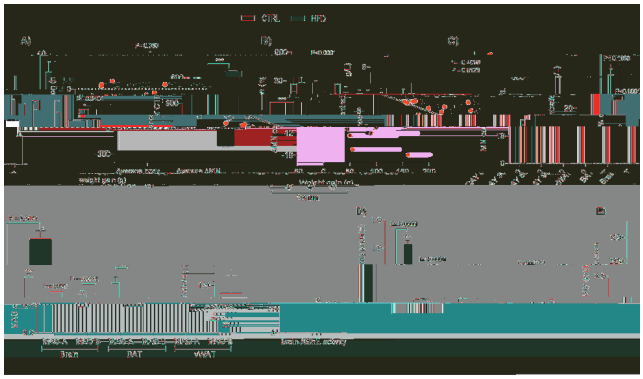

**Figure 2:** Effect of high fat diet on different molecular markers and enzyme activities in rats: [A] Metanephrine concentration (ng/L) in

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