Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.38609

Role of Paternal Small Non-Coding RNAs and Dopamine Signaling in High-Fat Diet-Induced Transgenerational Reward Memory

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Abstract. Parental environmental exposure can affect offspring through hereditary germline changes without changing the DNA sequence. Ongoing studies show that parental diet can induce heritable molecular changes, thereby affecting offspring metabolism, behavior, and neurological function. However, the mechanism behind these persistent neurobehavioral effects remains unclear. In order to solve this problem, this study explored whether the parent HFD will produce cross-generational memory, thereby changing the reward-related behavior of the offspring. Specifically, it studied the role of dopamine regulation and sperm small non-coding RNA in this process. F0 male mice received a control diet or a high-fat diet before mating, while F1 and F2 male mice maintained a normal diet. Reward-related behaviors will be evaluated through various hedonic preferences and reward learning tests. The functional contribution of dopamine will be evaluated at the molecular, behavioral, and electrophysiological levels by methods such as Neuropixels and high-performance liquid chromatography. Small RNA sequencing will identify persistent sperm small non-coding RNA candidates between generations. Ultimately, this study aims to determine how paternal HFD causes cross-generational changes in reward-related behaviors through dopamine regulation and sperm small non-coding RNA. These findings may contribute to a better understanding of how paternal diet shapes the transgenerational memory of neurobehavior and to identify this genetic causal reproductive mechanism.

Keywords: High-fat diet, Transgenerational memory, Reward behavior, Small non-coding RNA, Dopamine signaling

1. Background

1.1. Paternal high-fat diet and germline epigenetic inheritance

Growing evidence shows that parental nutrition can affect the phenotype of offspring independently of DNA sequence changes [1-4]. In rodents, paternal HFD and obesity is associated with cross-generational changes in offspring metabolism. This is often seen with changes in sperm epigenetic information, including DNA methylation, microRNA, and other small RNAs [5, 6]. Mechanistic studies have shown that sperm RNA can transmit diet-related information. For example, sperm

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tsRNA from HFD-fed males reproduced metabolic changes when injected into normal fertilized eggs, and disrupting Dnmt2-dependent RNA modification prevented these genetic effects [1, 7, 8]. Recently, dietary-responsive sperm mitochondrial tRNA has been shown to transfer from the father to the embryo during fertilization and affect the metabolism of offspring [9]. Taken together, these findings support sperm RNA as a reasonable vehicle for paternal dietary information, despite the fact that specific RNA classes and phenotypes transmitted vary among [10-14].

1.2. Parental diet, hedonic behavior, and dopamine reward circuits

The evidence linking parental diet to reward-related neural changes was strongest in the maternal overnutrition model, not in the paternal model. Maternal HFD exposure has been shown to alter presynaptic dopamine regulation in the nucleus accumbens and increase the motivation of offspring for fat reward [15]. In addition, lactational HFD exposure can cause reprogramming of dopaminergic neural circuits [16]. Sarker et al. demonstrated that maternal overnutrition can produce behavioral phenotypes similar to addiction and pleasure, and persist through paternal inheritance. These changes are accompanied by a decrease in striatal dopamine and an increase in D2 receptor density [17]. A follow-up study showed that sperm tsRNA transmitted these characteristics and metabolic phenotypes between generations [18]. These studies established a link between ancestral diet, germline RNA, dopamine signaling, and reward behavior. However, they did not directly test whether paternal HFD alone initiated the same reward-circuit response.

1.3. Dopamine regulation as a candidate mechanism

The mesolimbic dopamine system, especially the ventral tegmental area (VTA) and nucleus accumbens (NAc), plays a central role in reinforcement, motivation, and reward learning. Tyrosine hydroxylase (TH), dopamine transporters (DAT) and D1 and D2 dopamine receptors can affect the synthesis, reuptake, and signal transduction of dopamine. Transgenerational overnutrition models usually show a hypodopaminergic state, paired with increased sensitivity to reward stimuli [17, 19]. This makes dopamine regulation a powerful candidate mechanism for explaining reward-related behavioral changes. Recent studies have found that neurons associated with D1 and D2 have different reward responses [20]. This provides a framework for examining whether transgenerational dietary effects alter the balance of these neuronal populations.

1.4. Present study

This work investigated whether paternal HFD established transgenerational memory in offspring. It will focus on whether dopamine signaling and germline small non-coding RNA (sncRNA) contribute to changes in reward-related behaviors. Using control and HFD paternal lineages across F1 and F2 generations, inherited changes in reward-related behavior will first be characterized. The contribution of altered dopamine signaling and reward-circuit neuronal activity to these behavioral phenotypes will then be determined. Finally, sperm small non-coding RNA will be analyzed between generations to determine persistent molecular changes that may be responsible for transmission. In summary, these experiments were designed to discover the relationship between parental dietary exposure, germline epigenetic information, dopamine regulation, and offspring reward behavior. The overall workflow is shown in Figure 1.

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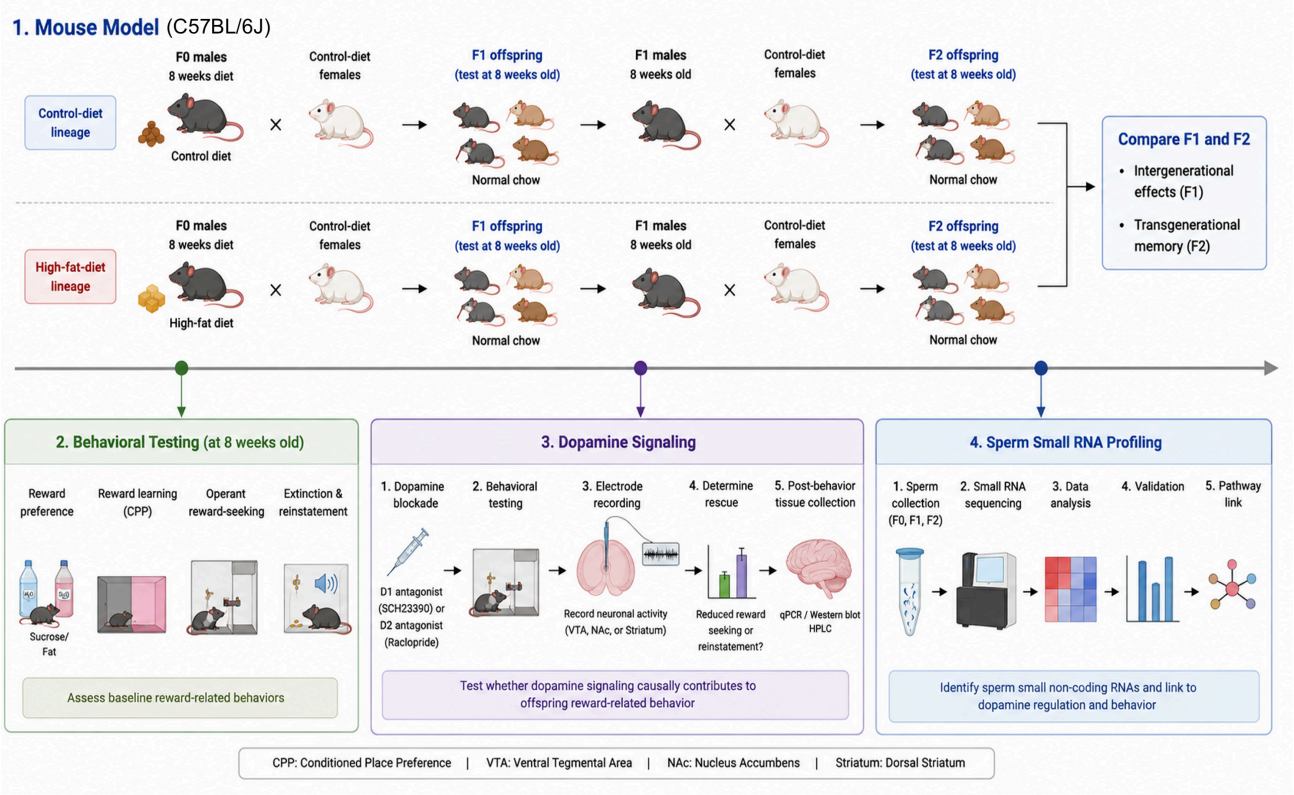


Figure 1. Graphical outline of the mouse models and main experiments

2. Project description

2.1. Research question

Does paternal high-fat diet create a transgenerational memory that alters offspring reward-related behavior through dopamine regulation?

2.2. Hypothesis

It is hypothesized that paternal HFD alters the sperm sncRNA profile, creating an inherited molecule that can reprogram dopamine regulation in offspring reward circuits. This altered dopaminergic state increases reward sensitivity, compulsive reward seeking, and reinstatement in F1 offspring, and persists into F2 offspring, although there is no direct HFD exposure. Based on previous dietary genetic studies, persistent tRNA-derived small RNAs were further predicted to be the carrier that connects primitive exposures with the transgenerational phenotype [7, 8, 10, 18, 21].

2.3. Mouse model and experimental design

Adult F0 male C57BL/6J mice will be divided into control-diet and HFD groups (n=10/group) and maintained on the corresponding diet for 8 weeks before mating with control-diet females. All F1 offspring will be maintained on normal chow and tested at 8 weeks of age. Eight-week-old F1 males will then be bred with control-diet females to generate F2 offspring, which will also remain on normal chow and be tested at 8 weeks. Thus, the original HFD exposure is restricted to the F0 generation. Control-lineage and HFD-lineage offspring will be compared within each generation. F1

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changes will represent intergenerational effects, whereas persistence of phenotypes into F2 will support a transgenerational memory of the original paternal exposure [2].

3. Objective 1: determine whether paternal high-fat diet alters offspring reward-related behavior

3.1. Rationale for assessing reward behavior

If paternal HFD changes offspring reward systems, HFD-lineage offspring should differ from controls across multiple levels of reward processing. Previous dietary studies have reported altered palatable-food motivation and transgenerational hedonic or addiction-like behaviors [15, 17, 19]. Behavioral changes will therefore be assessed from simple preference to reward learning, punishment-resistant responding, and relapse-like reinstatement.

3.2. Behavioral assessment methods

3.2.1. Sucrose/fat preference test

Mice will receive simultaneous access to a palatable reward, such as sucrose solution or high-fat/high-sugar food, and a control option. Consumption and preference will be quantified to measure baseline hedonic reward sensitivity.

3.2.2. Conditioned place preference

Conditioned place preference (CPP) will assess reward-associated learning. One chamber will be paired with a palatable reward, and a second chamber will be an unpaired control environment. The rate of preference development and final strength of preference for the reward-paired chamber will be compared between groups.

3.2.3. Operant reward-seeking

Mice will be trained to lever press to obtain a palatable reward. A mild foot shock will then be introduced as a negative consequence associated with reward seeking. Motivation will be assessed using response rates and breakpoint. Continued responding despite punishment will be used as a measure of compulsive-like reward seeking.

3.2.4. Extinction and reinstatement

Following acquisition of reward-seeking behavior, the reward will be withheld until responding declines. A small reward prime will then be presented, and recovery of responding will be quantified as a measure of reinstatement and relapse-like reward seeking.

3.3. Expected behavioral outcomes

If paternal HFD increases inherited reward sensitivity, HFD-lineage F1 offspring are expected to show stronger palatable-reward preference, faster or stronger CPP, greater operant responding and breakpoint, greater punishment-resistant responding, and stronger reinstatement after extinction. If comparable changes persist in F2, the behavioral findings would support transgenerational memory. If the phenotype is present only in F1, the findings would be more consistent with an

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intergenerational effect. These predictions extend the hedonic phenotypes observed in ancestral overnutrition models [17, 18] to a paternal-HFD paradigm.

4. Objective 2: assess whether paternal high-fat diet changes dopamine regulation in offspring reward circuits

4.1. Rationale for investigating dopamine signaling

The behavioral phenotype proposed in Objective 1 may result from altered dopamine regulation within mesolimbic and striatal reward circuits. Prior parental-diet studies have documented changes in NAc dopamine regulation, dopaminergic circuit development, striatal dopamine concentration, and D2 receptor density [15-17, 19]. This objective will test whether similar changes occur after paternal HFD and whether dopamine receptor signaling causally contributes to the inherited behavioral phenotype.

4.2. Dopaminergic and electrophysiological assessment methods

4.2.1. Dopamine receptor manipulation and behavioral retesting

F1 and F2 offspring from control and HFD lineages will first be characterized behaviorally as described in Objective 1. Dopamine receptor signaling will then be blocked pharmacologically before retesting. D1 receptor signaling will be blocked with SCH23390 and D2 receptor signaling with raclopride. Operant reward-seeking and extinction/reinstatement assays will be repeated under treated and untreated conditions. A selective reduction of the HFD-lineage phenotype after receptor blockade would indicate that dopamine signaling contributes functionally to the behavioral change.

4.2.2. Neuropixels electrophysiological recording

Neuropixels electrodes will record populations of neurons during reward-seeking behavior in the VTA, NAc, and striatum. Analyses will compare the proportions of reward-responsive neurons, firing rates during reward-predictive cues, and the distribution of neuron types and different neuronal responses between lineages. HFD-lineage offspring are expected to show a more reward-biased population distribution, characterized by a greater proportion of reward-activated neurons, fewer reward-suppressed neurons, and increased firing in response to reward-predictive cues. Such an imbalance may indicate altered neural encoding of reward value, in which reward-related signals are disproportionately represented and may contribute to enhanced reward-seeking behavior. Recordings after dopamine receptor blockade will determine whether this imbalance is dopamine-dependent and shifts toward the control distribution.

4.2.3. Dopamine-related gene expression

After behavioral and electrophysiological testing, the VTA, NAc, and striatum will be collected. qPCR will quantify Th, Slc6a3, Drd1, and Drd2 expression, representing dopamine synthesis, transport, and receptor signaling.

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4.2.4. Dopamine-related protein expression

Western blotting will quantify TH, DAT, D1R, and D2R protein expression in VTA, NAc, and striatum. Protein expression levels will be matched and assessed against their corresponding transcript levels.

4.2.5. Dopamine quantification by HPLC

High-performance liquid chromatography (HPLC) will quantify dopamine concentrations in the NAc and striatum. These measurements will determine whether the molecular changes are associated with altered dopamine levels in major reward-related regions.

4.3. Expected dopaminergic and neuronal outcomes

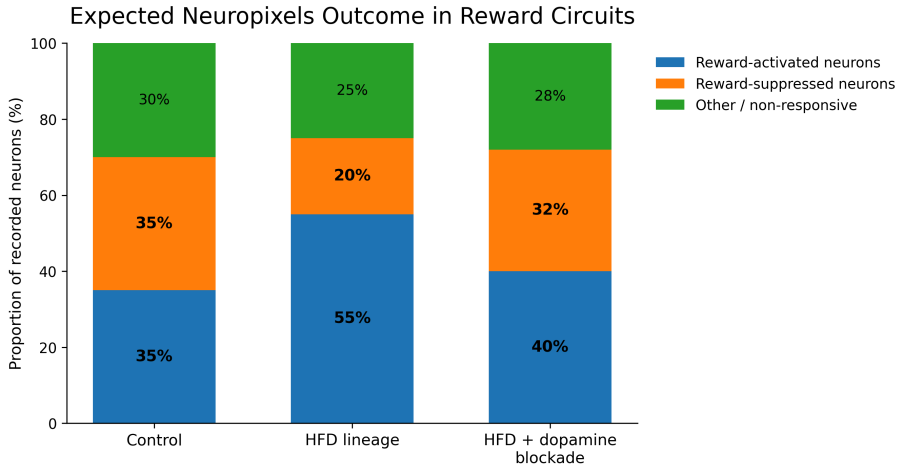


Figure 2. Example Outcomes of Neuropixel Recording

HFD-lineage offspring are expected to show dopamine dysregulation with stronger reward-seeking behavior. On the molecular level, expression of Th and Slc6a3 may decrease, thereby reducing TH and DAT protein levels. This may be accompanied by compensatory changes in D1R/D2R and lower dopamine concentrations in the NAc and striatum. Overall, HFD-lineage offspring should establish a hypodopaminergic pattern that is observed in previous transgenerational overnutrition models [17]. As in Figure 2, Neuropixels recordings will show a greater proportion of reward-activated neurons, higher firing rates during reward-predictive cues, and fewer neurons opposing reward-seeking responses. The receptor blockade assay should reduce reward-activated firing, decrease the overrepresentation of reward-promoting neurons, and reduce compulsive reward-seeking behaviors. Aligned neuronal and behavioral results would provide stronger evidence that dopamine signaling contributes to the inherited behavioral changes.

5. Objective 3: identify whether germline small non-coding RNAs are altered by paternal high-fat diet and may contribute to transmission

5.1. Rationale for investigating germline sncRNAs

Sperm sncRNAs have been shown to transmit paternal dietary information and behavioral memories [10-13]. Paternal HFD and obesity have been associated with altered sperm miRNAs across

DOI: 10.54254/2753-8818/2026.38609

generations [2, 5, 6, 21, 22], while other studies have shown that sperm tsRNAs can reproduce or prevent diet-induced offspring phenotypes [1, 7, 8, 10]. The 2019 study by Sarker et al. specifically connected sperm tsRNAs to transgenerational hedonic behavior [18]. This experiment will profile multiple sncRNA classes and narrow down the strong mechanistic candidate.

5.2. Sperm sncRNA profiling and analysis methods

5.2.1. Sperm collection and small RNA extraction

Mature sperm will be collected and purified from F0, F1, and F2 males of the two lineages. The purified sperm will be lysed, and the total RNA will be isolated using a silica membrane spin-column extraction method. This allows the capture of miRNA, tRNA-derived small RNA, piRNA, and other short RNA fragments [11, 12].

5.2.2. Small RNA sequencing and classification

Small RNA libraries will be prepared using an adapter-ligation-based method. The RNAs will then be reverse transcribed to cDNA, amplified by PCR, and sequenced. Reads will be mapped to the mouse small RNA reference databases, then classified into miRNAs, tRNA-derived small RNAs, piRNAs, or other small RNA populations [11, 21].

5.2.3. Differential expression analysis across generations

Within each generation, sperm RNA expression between the control and HFD lineages will be compared. Significantly increased and decreased sncRNAs will be identified for each generation. These altered RNAs will then be compared across generations to distinguish persistent changes.

5.2.4. Candidate validation and pathway association

The selected candidate sncRNAs will be verified by qPCR to confirm the repeatability of the expression differences obtained by sequencing. Then the predicted target genes will be subjected to pathway enrichment analysis, focusing on the field of dopamine signaling and reward processing. The candidate RNA pattern will be compared with the dopamine markers and behavioral test results measured in Objectives 1 and 2.

5.3. Expected germline sncRNA outcomes

Sperm sncRNA expression is expected to differ between HFD-lineage and control males. Based on transgenerational hedonic models, tRNA-derived small RNAs are predicted to show particularly strong biologically relevant changes [7, 8, 10, 18, 21]. A subset of altered tsRNAs is expected to persist across F0, F1, and F2, whereas changes restricted to F0 would be more consistent with a direct response to dietary exposure. qPCR validation should reproduce the sequencing-derived direction of change, and pathway enrichment is expected to identify target genes involved in dopamine signaling, reward processing, and synaptic plasticity. Persistent tsRNA changes that coincide with altered dopamine markers and reward behavior would represent the strongest candidates for a germline mechanism of transgenerational memory.

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6. Conclusion

This proposal integrates behavioral, neurobiological, and germline analyses to test whether paternal HFD can establish an inherited reward-related phenotype. Current literature supports two components of the model: paternal dietary exposures can alter sperm RNA and transmit offspring phenotypes [1-13, 21, 22], and ancestral or maternal HFD can alter reward behavior and dopamine signaling across generations [15-19]. The unanswered question is whether paternal HFD alone can cause transgenerational changes in dopamine-related reward behavior. Objective 1 assessed the baseline of behavioral phenotypes. Objective 2 examined the role of dopamine signaling, while Objective 3 identified persistent changes in sperm sncRNAs. However, it should be acknowledged that the study design does not completely eliminate the potential maternal or developmental effects on offspring phenotypes. Future research should focus on establishing the causal role of candidate sncRNAs in transmission. Consistent candidate genes can be introduced into control fertilized eggs to test whether they can replicate dopamine and behavioral phenotypes. Additional inhibition or depletion tests can determine whether they are necessary for transmission. The effects of sperm sncRNAs on early embryonic gene expression and dopamine circuit development can also be detected.

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