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Partial Knockout of the Dopamine Transporter Gene in Rats Alters the Haematological Profile and Erythrocyte Sensitivity to Chronic Ethanol Exposure: A Pilot Study

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Abstract

Background/Objectives: Chronic ethanol consumption damages erythrocyte membranes, reducing their deformability and disrupting erythropoiesis. Dopaminergic signalling plays an important role in regulating haematopoietic stem cells. However, the impact of genetic alterations in the dopamine system on the functional properties of erythrocytes and their resistance to the toxic effects of alcohol remains understudied. In this study, we evaluated haematological parameters and the sensitivity of erythrocytes to chronic ethanol exposure in DAT-HET rats (heterozygous for the dopamine transporter gene) compared to Wistar rats. **Methods**: Both lines of rats were subjected to chronic alcoholisation (10% ethanol for six months). Flow cytometry was used to assess reticulocyte production, intracellular esterase activity (cell viability), and membrane lipid asymmetry disruptions. Laser diffraction was used to evaluate osmotic resistance (deformability). **Results**: The partial knockout of the DAT gene significantly altered the baseline haematological profile, resulting in pronounced leukopenia, moderate thrombocytopenia, moderate erythrocyte macrocytosis, and reduced intracellular esterase activity. The consequences of chronic ethanol consumption in Wistar rats (anaemia, decreased reticulocyte count, increased osmotic rigidity of erythrocytes, and an increased proportion of annexin-positive cells) were similar to those observed in humans, thus confirming the validity of the experimental model. In contrast, chronic alcohol exposure did not significantly influence haematopoiesis parameters or erythrocyte biophysical properties in DAT-HET rats, indicating that even a 50% loss of the dopamine transporter strongly prevented ethanol's damaging effects on blood cells. **Conclusions**: The marked contrast between Wistar rats (which develop alcohol-induced anaemia and erythrocyte dysfunction similar to humans) and DAT-HET rats (which remain largely unaffected) demonstrates that dopaminergic signalling plays a previously unrecognised role in determining erythrocyte sensitivity to alcohol toxicity.

Keywords: DAT-HET rats; Wistar rats; chronic ethanol consumption; erythrocytes; deformability

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Academic Editors: Sigrun Lange and Jameel M. Inal

Received: 17 April 2026
Revised: 12 May 2026
Accepted: 15 May 2026
Published: 19 May 2026

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1. Introduction

The most common and earliest manifestation of alcohol dependence is the development of anaemia, accompanied by morphological abnormalities of erythrocytes (i.e., anisocytosis and poikilocytosis), moderate thrombocytopenia, and alterations in leukocyte subpopulation ratios [1]. Profound metabolic changes during alcohol intoxication cause the abnormalities detected in the haemogram analysis. Chronic alcohol consumption leads to disturbances in erythrocyte membranes, notably abnormal micro-organisation of the membrane surface and a decrease in sialic acid content. This results in changes to the surface charge, increased aggregation, and impaired microcirculation [2]. Disruptions in the structure and function of erythrocyte cytoskeletal membrane proteins, along with changes in the density distribution of key protein complexes and a decrease in ankyrin levels, accompanied by an increased density of Band 3, proteins 4.2 and 4.9, have been identified in patients with alcohol dependence [3].

One of the main causes of disruption is an alteration in membrane microviscosity (i.e., fluidity), due to increased cholesterol accumulation and an imbalance between saturated and unsaturated fatty acids [4–7]. All these alterations lead to an increased elimination of functionally impaired erythrocytes, which is considered to be one of the causes of anaemia associated with alcohol intoxication.

Deformability is a primary factor in determining erythrocyte lifespan; a reduction in deformability increases sequestration and destruction of erythrocytes in the spleen [8]. Erythrocyte deformability enables passage through narrow capillaries without loss of cellular integrity, ensuring maximum possible contact between the erythrocyte membrane and capillary endothelium. This contact is necessary for efficient gas exchange [8,9]. Erythrocyte deformability mainly depends on cytoskeletal proteins (spectrin, actin, ankyrin, band 4.1, and band 3 proteins) [10], intracellular viscosity (haemoglobin concentration), and excess membrane surface area [8]. Shape abnormalities (e.g., spherisation and dysmorphia) and impaired mechanical properties (e.g., rigidity and loss of excess membrane area) reduce contact area and, consequently, the efficiency of gas exchange [9]. Abnormalities in erythrocyte membrane lipid composition, which lead to altered membrane fluidity [11] and the reorganisation of cytoskeletal proteins [3], are the primary causes of impaired deformability in alcoholism. Reduced erythrocyte deformability in patients with alcohol dependence complicated by liver cirrhosis causes disturbances in erythrocyte transit through microchannels that mimic the capillary network: transit speed decreases, and a high percentage of occlusions are observed, indicating impaired microrheology [12].

Neurons are the cells in the body that depend most on energy [13]. Red blood cells supply neurons with the oxygen necessary for their exceptionally high ATP production. Dysfunction of red blood cells, such as impaired deformability and rheology, directly impairs oxygen delivery to the brain. This can cause energy deficiency, neuroinflammation, and neuronal dysfunction [8,13]. Although neurodegeneration has historically been considered an issue exclusive to neurons, the relationship between neurodegeneration and the functional state of red blood cells has recently attracted increasing attention. For example, exposure to beta-amyloid decreases the deformability of human erythrocytes in Alzheimer's disease (AD), making their membranes stiffer. Moreover, increased erythrocyte rigidity is exacerbated in AD patients by low levels of nitric oxide (NO), which is necessary for vasodilation [14]. These conditions create a vicious cycle: reduced perfusion in the brain's microvasculature due to impaired erythrocyte deformability creates conditions for chronic brain hypoxia. This, in turn, accelerates the accumulation of amyloid plaques (APP is synthesised in response to hypoxia) and neuronal death.

Parkinson's disease is a chronic and progressive neurological disorder characterised by the loss of dopaminergic neurons in the substantia nigra. Systemic inflammation is a

well-known feature of Parkinson's disease and many other degenerative disorders [15]. Increased permeability of the blood–brain barrier and cerebral vascular dysfunction contribute to the onset of systemic inflammation. This affects the coagulation system, promotes the oxidative modification of erythrocyte membranes (increasing their rigidity and altering their charge), and enhances the process of programmed erythrocyte death (eryptosis). Consequently, there has been a proposal to assess eryptosis levels as a marker of Parkinson's disease progression [16]. A histopathological feature of many neurodegenerative diseases, including Parkinson's disease, is abnormal cytoskeletal organisation, in which dopaminergic signalling plays a significant regulatory role in structures involved in synapse integrity and function [17]. The neuronal cytoskeleton and the erythrocyte membrane share some identical structural proteins; for example, the key cytoskeletal protein band 4.1 [18]. This suggests that disruptions in dopaminergic signalling may affect not only the membranes of nerve cells, but also the cytoskeletal protein organisation of other cells, including erythrocytes.

Dopamine is traditionally considered a key neurotransmitter in the central nervous system, where it regulates motor activity, motivation, and endocrine functions. The dopamine transporter (DAT) protein is located in the presynaptic membrane of neurons. Its primary function is to capture the dopamine that is released into the synaptic cleft, returning it to the neuron in order to terminate the signal and enable its reuse [19]. Pathologies associated with enhanced dopaminergic signalling include schizophrenia, psychosis, mania, addiction, and hyperkinetic movement disorders such as Tourette's syndrome [20]. A convenient model for studying such conditions is the genetic modification of the dopaminergic signalling system [21]. Initially, DAT-KO mice were created; however, due to their extreme phenotype, which can affect or even worsen some parameters, DAT-KO mice were abandoned in favour of heterozygous hypofunctional DAT gene knockout mice (DAT-HET). The behavioural phenotype of these animals retained motor hyperactivity, attention deficit, impulsive behaviour, and amphetamine-mediated recovery, which is consistent with attention deficit hyperactivity disorder (ADHD) [22]. Subsequently, DAT-KO and DAT-HET rat models were developed, whose larger brains facilitate surgical manipulation and electrophysiological recordings, and whose physiology closely resembles that of humans [21,23].

Importantly, dopamine plays a significant role in regulating haematopoiesis. Studies have shown that signalling via D2-subfamily receptors (D2 and D3) is necessary for maintaining the pool of haematopoietic stem and progenitor cells (HSPCs) in the bone marrow [24,25]. Although erythrocytes are terminally differentiated, enucleated blood cells, their functional characteristics (deformability, osmotic resistance, and oxygen transport function) and the process of erythropoiesis itself may depend on dopaminergic regulation at the early stages of haematopoiesis [26]. D2-type receptors are crucial for haematopoiesis in the bone marrow, spleen, lymph nodes, and thymus. Genetic deletion of D2-type receptors in haematopoietic cells disrupts HSPC proliferation and reduces blood cell production in response to lipopolysaccharide stimulation, particularly in the bone marrow and spleen [27]. The dopamine D2 receptor and the dopamine transporter modulate each other's functions. The presynaptically expressed splice isoform of the D2S receptor interacts with DAT and stabilises it in the plasma membrane [28]. Therefore, D2S levels influence the level of functional DAT localised on the cell membrane. Currently, there are no data in the literature on the effect of the dopamine transporter on haematopoiesis. However, the effect of DAT gene knockout on bone tissue (decreased bone density and bone mass in DAT gene knockout mice) suggests impaired haematopoietic stem cell activity [29].

Ethanol exerts its toxic effects by altering numerous neurotransmitter systems, including those involving serotonin, dopamine, gamma-aminobutyric acid, glutamate, acetyl-

choline, and opioids [30]. Dopamine plays a key role in substance use disorders, including alcohol, via various neural circuits involved in compulsive behaviour, reward systems, and habit formation [31]. Alcohol dependence accelerates neurodegeneration by impairing the brain's ability to clear toxic proteins, disrupting neurotransmitter balance, and exacerbating inflammation and oxidative stress. Dysfunction of central dopaminergic neurotransmission plays a role in the pathogenesis of schizophrenia, as well as drug and alcohol dependence [32]. Long-term alcohol exposure may accelerate neurodegeneration by modulating key pathological features of Alzheimer's disease [33]. ADHD and alcohol-use disorder share genetic, neurobiological, and neuropsychological links [34].

A genetic defect in dopamine reuptake (DAT knockout) alters dopamine signalling in the haematopoietic and progenitor niches of the bone marrow [35]. We hypothesise that impaired dopamine signalling in DAT-heterozygous rats may lead to altered erythrocyte membrane properties, affecting erythrocyte resistance to the damaging effects of chronic ethanol compared to Wistar rats. This assessment is of interest because dopamine receptor antagonists (neuroleptics) are widely used in clinical practice, yet their relationship with red blood cell parameters remains poorly understood. In this study, we demonstrated that, unlike Wistar rats, chronic alcohol exposure did not significantly influence haematopoiesis parameters in DAT-HET rats, suggesting that the dopamine transporter plays a critical role in alcohol's effects on blood cells.

2. Results

2.1. Complete Blood Count

A complete blood count was performed to evaluate the health of the rats and assess the impact of the targeted mutation of the dopamine transporter gene on circulating cell counts. The results revealed that the two groups, DH and W, differed in several parameters, with the primary difference being significantly lower levels of all leukocyte cells in DAT-HET rats.

2.1.1. Leukocytes

The analysis of different leukocyte subpopulations was based on three main fractions: lymphocytes (LYM), granulocytes (GRAN, primarily neutrophils), and mid-range cells (MID, including monocytes, eosinophils, basophils, and their precursors). Wistar rats had significantly higher levels of leukocytes than DAT-HET rats (8.88 ± 2.13 vs. $2.88 \pm 1.04 \times 10^{12}$/L), with higher numerical values observed in all three leukocyte fractions (see Figure 1). Chronic alcohol consumption in Wistar rats resulted in a decrease in circulating lymphocyte levels ($3.61 \pm 1.30 \times 10^{12}$/L, $p \leq 0.02$), leading to a state of moderate lymphocytopenia. A decrease in granulocyte levels was also noted ($p \leq 0.019$).

The absolute leukocyte count in the DAT-HET rat was 3.3 times lower, with lymphocyte and granulocyte counts 3.1 and 2.7 times lower, respectively, than in Wistar rats (Figure 1a). Differences were also found in the distribution of leukocyte subpopulations between rat groups (Figure 1d–f). Chronic alcohol exposure affected the concentration of leukocyte subpopulations in DAT-HET rats to varying degrees. Alcoholisation did not alter the level of circulating lymphocytes (1.91 ± 0.71 vs. $1.73 \pm 0.78 \times 10^{12}$/L, $p \leq 0.72$), but decreased the concentration of granulocytes (Welch *t*-test: DH vs. DHA, 0.88 ± 0.36 vs. $0.53 \pm 0.25 \times 10^{12}$/L, $p \leq 0.026$).

The results indicate that even a partial knockout of the dopamine transporter gene can significantly reduce the concentration of white blood cells (WBCs) in the blood.

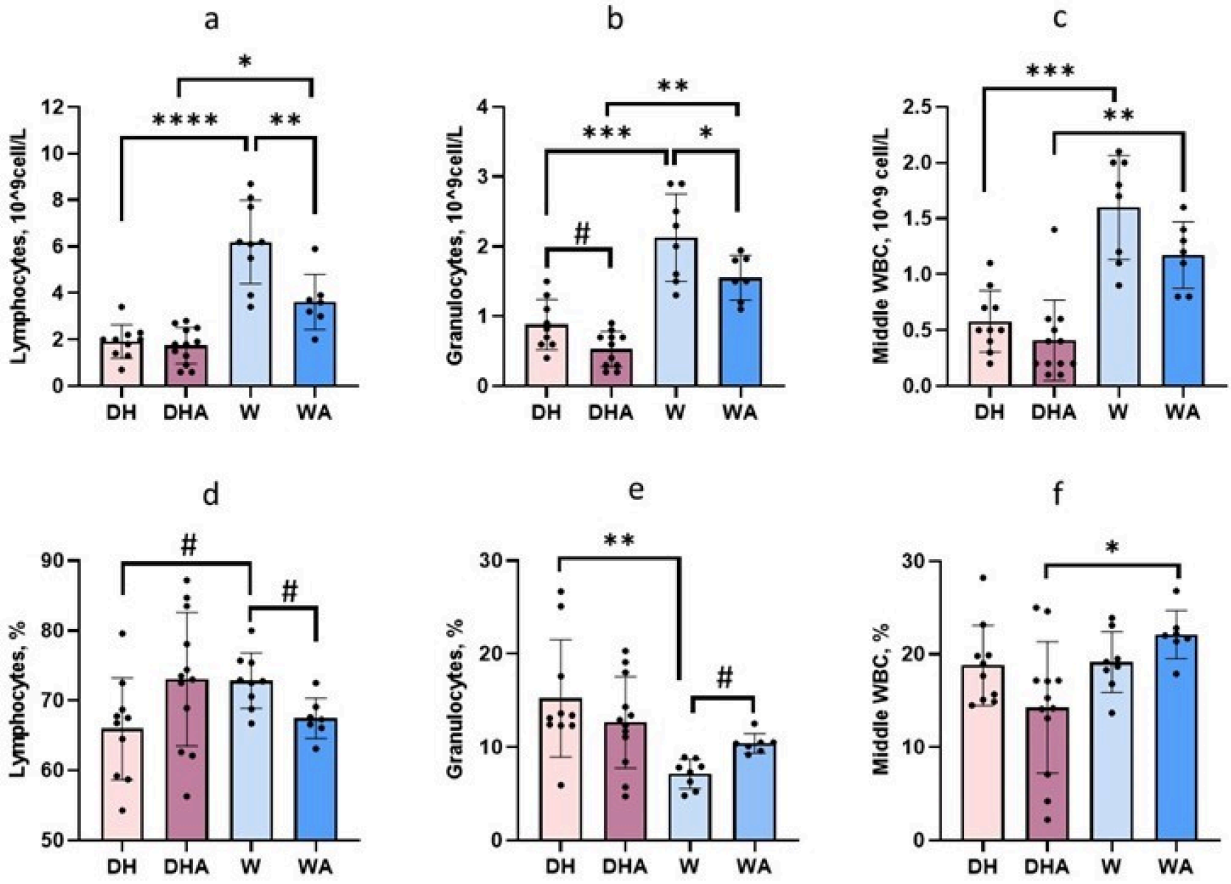


Figure 1. Leukocyte indices in the haematogram of Wistar and DAT-HET rats subjected to chronic alcohol exposure. The upper panel shows the absolute values of the following leukocyte subpopulations: (**a**)—Lymphocyte count; (**b**)—granulocyte count; and (**c**)—mid-range leukocyte count, according to the distribution on the Medonic-M20 haematology analyser. The lower panel shows the percentage content of leukocyte subpopulations: (**d**)—Lymphocyte, %; (**e**)—Granulocytes, %; (**f**)—Mid-range leukocytes, %. Data are presented as M ± SD; ANOVA statistics: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$ and ****, $p \leq 0.0001$; Welch *t*-test statistics: #, $p \leq 0.05$.

2.1.2. Platelets

Since thrombopoiesis, like red blood cell production, is a line of myeloid haematopoiesis, it was important to evaluate how these parameters respond to chronic alcohol consumption. In Wistar rats, chronic alcohol consumption resulted in a decrease in platelet count ($p \leq 0.007$) (Figure 2a) and an increase in mean platelet volume (MPV) ($p \leq 0.061$), due to an increase in the proportion of large platelets in circulation (LPCR; large platelet ratio, $p \leq 0.02$; Figure 2b). This led to an increase in PDW (platelet distribution width; Figure 2c). At baseline, DAT-HET rats exhibited moderate thrombocytopenia ($p \leq 0.006$) compared to Wistar rats. Exposure to alcohol in heterozygous DAT-HET rats did not significantly affect platelet counts ($p \geq 0.37$) or the level of the large platelet subset ($p \geq 0.34$) (Figure 2a,b). Regardless of alcohol consumption, scatter plots showed that values in heterozygous rats were consistently higher than in Wistar rats.

The mechanism by which LPCR increases in alcoholism remains unclear, but it has been hypothesised that the increase in the proportion of large platelets is due to changes in the cytokine balance in the bone marrow niche, as well as to disruption of cytoskeleton reorganisation and microtubule and actin rearrangement during megakaryocyte maturation [36]. The LPCR parameter is considered a prognostic marker for haemostatic disorders and an independent predictor of disease severity, for example, infarctions, liver cirrhosis, and outcomes during resuscitation [37].

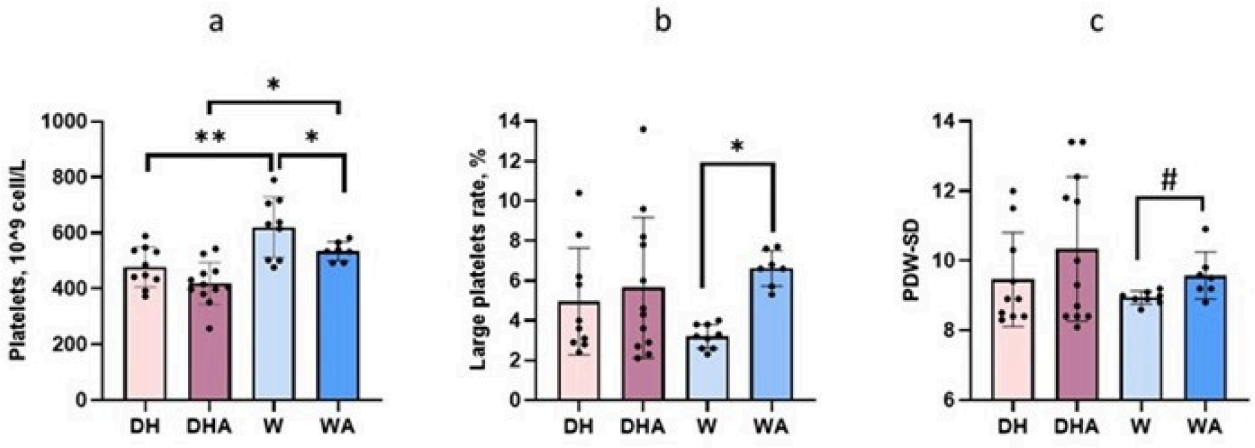


Figure 2. Platelet parameters in rats from two genetic linesduring chronic alcohol consumption: (**a**)—Platelet count; (**b**)—Large platelet ratio; and (**c**)—Platelet distribution width. Medonic-20 M haematology analyser. Data are presented as M ± SD; ANOVA statistics: *, $p \leq 0.05$, **, $p \leq 0.01$; Welch *t*-test W vs. WA: #, $p \leq 0.05$.

2.1.3. Erythrocytes

The baseline level of circulating erythrocytes in DAT-HET rats was lower than in Wistar rats (Welch's *t*-test: W vs. DH = 8.55 ± 1.21 vs. 7.98 ± 0.69 × 10^{12}/L, $p \leq 0.023$; see Figure 3a), whereas haemoglobin concentration was higher due to increased MCH (mean corpuscular haemoglobin) levels (Welch's *t*-test: W vs. DH = 155 ± 8 vs. 149 ± 6 g/L, $p \leq 0.14$; see Figure 3c). Similarly, mean corpuscular volume (MCV), which, combined with the other parameters, indicates early macrocytic changes upon partial knockout of the dopamine transporter, was significantly higher ($p \leq 0.001$; Figure 3b) in DAT-HET rats than in Wistar rats.

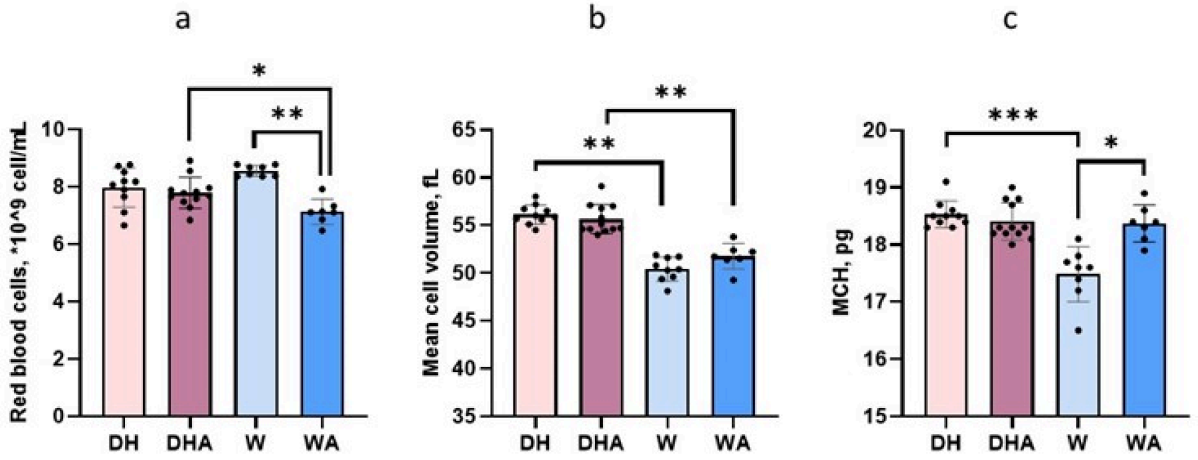


Figure 3. Erythrocyte parameters in rats of two genetic groups during chronic alcohol consumption: (**a**)—Red blood cell count (RBC); (**b**)—Mean corpuscular volume (MCV); and (**c**)—Mean corpuscular haemoglobin (MCH). Medonic-20 M haematology analyser. Data are presented as M ± SD; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

According to the Welch *t*-test, chronic alcohol exposure did not result in a significant change in erythrogram parameters in DAT-HET rats; the differences between groups of heterozygous rats with and without alcohol consumption were not statistically significant (Figure 3a–c). By contrast, the WA group showed a significant decrease in erythrocyte count ($p \leq 0.007$; Figure 3a) and an increase in MCH (Figure 3c).

Reticulocytes are immature erythrocytes that are released into the circulation daily to replace old cells. As in humans, erythropoiesis in rats predominantly occurs in the bone marrow [38]. The level of reticulocytes serves as a key indicator of erythropoiesis, reflecting the balance between the elimination of old erythrocytes and the proliferative activity of the bone marrow. Erythropoiesis is more intense in rats than in humans because the lifespan of circulating erythrocytes is approximately twice as short, at 59.8 ± 2.2 days in Wistar rats [39]. The absolute reticulocyte concentration (Figure 4c) and their overall percentage in the circulating pool were equal in both rat groups (DH vs. W: 5.13 ± 0.19 vs. 5.25 ± 0.71%, $p < 0.68$), although the fractional composition of reticulocytes differed—the percentage of the young reticulocyte subpopulation was higher in Wistar rats than in heterozygous rats (Figure 4a).

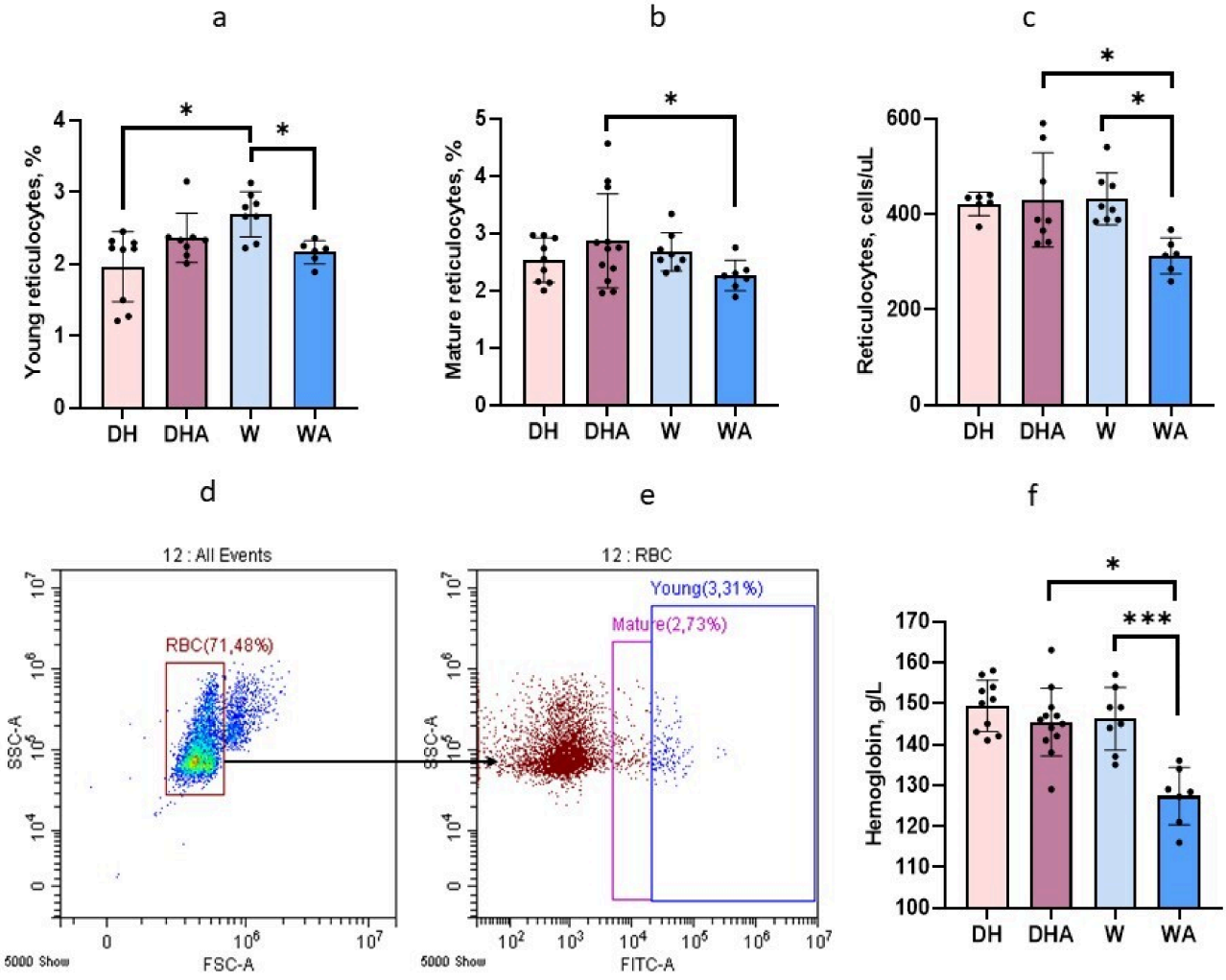


Figure 4. Indicators of reticulopoiesis and haemoglobin status in rats. (**a**)—Proportion of young reticulocyte forms with high fluorescence level; (**b**)—Proportion of mature reticulocyte forms with low fluorescence level; (**c**)—Absolute reticulocyte count in blood; (**d**)—Example of region of interest (gating) for erythrocytes in whole blood; (**e**)—Example of reticulocyte subpopulation registration; (**f**)—Haemoglobin levels in rats (haematology analyser). The black arrow shows that the red blood cell gate has been selected. Data obtained by flow cytometry using thiazole orange staining of whole blood cells; data presented as M ± SD; *, $p \leq 0.05$; ***, $p \leq 0.001$.

Chronic alcohol consumption in Wistar rats resulted in reduced reticulocyte release into circulation. Assessment of differences in the level of mature reticulocytes showed a significant difference between the W and WA groups (Welch's *t*-test, $p \leq 0.021$).

Consequently, the absolute reticulocyte count in alcohol-exposed Wistar rats decreased (Figure 4c). Correspondingly, a decrease in erythrocyte concentration and a significant decrease in haemoglobin concentration were observed (Figure 4e,f). A decrease in the absolute reticulocyte count, primarily due to reduced production of immature cells (Figure 4a), is considered an indicator of suppressed erythropoiesis in the bone marrow during alcohol intoxication [1].

In contrast, none of the changes in reticulocyte parameters in DAT-HET rats exposed to alcohol were significant (ANOVA), partly due to high dispersion of values. Assessment by Welch's *t*-test indicated that heterozygous rats still showed a trend towards increased production of young reticulocytes upon alcohol exposure ($p \leq 0.067$), which, given the small group size, can be considered a trend.

Based on the identified erythrogram abnormalities in Wistar rats, chronic alcohol consumption was found to lead to the development of alcohol-induced anaemia due to significant suppression of reticulopoiesis (see Figure 4). In DAT-HET rats exposed to alcohol, erythrogram parameters did not exhibit any statistically significant abnormalities. However, the observed trend of increased production of young reticulocytes does not allow us to definitively conclude that six months of alcohol consumption does not affect erythropoiesis in this group.

2.2. Evaluation of Rat Erythrocytes by Laser Diffraction–Osmotic Fragility Test (OFT)

The baseline osmotic resistance of the erythrocytes, as determined by the osmolality of the buffer at which 50% of the erythrocytes lysed (H50), was not different between the Wistar and DAT-HET rats (Welch's *t*-test: DH vs. W: 130.1 ± 4.6 vs. 132.0 ± 4.8%, $p \leq 0.23$; see Figure 5a). This value corresponded with the previously obtained H50 values for the Wistar rats [40].

Chronic alcohol exposure caused an increase in the osmotic rigidity of erythrocytes in Wistar rats. On average, lysis of 50% of erythrocytes in the WA group was achieved at a buffer osmolality 10 mOsm lower than in the W group. This type of osmotic resistance, or rigidity as opposed to fragility, is considered a disturbance to cell membranes that leads to decreased deformability [41]. Levels of osmotic lysis at 150 mOsm (reaction onset) and 100 mOsm (lysis completion indicator) were lower in rats that consumed alcohol than in Wistar rats (see Figure 5c,d). The H90-H10 parameter characterises the heterogeneity of the population with regard to the biophysical properties of erythrocyte membranes when withstanding hypo-osmotic stress; the higher the value (i.e., the wider the H90-H10 range), the more heterogeneous the cell population. As alcohol exposure in Wistar rats resulted in increased erythrocyte rigidity in response to hypo-osmotic stress across the entire tested range of osmolalities, no widening of the H90-H10 range was observed (W vs. WA: 52.1 ± 1.9 vs. 51.2 ± 1.0 mOsm, $p \leq 0.77$). At the same time, alcohol exposure did not significantly affect the osmotic test parameters in DAT-HET rats (see Figure 5).

During hypo-osmotic stress, water enters the cell, and the erythrocytes lose their discocytic shape and become spherical. However, membrane stretching prevents lysis up to a certain point. The ability of the membrane to swell without rupturing is provided by the excess membrane area relative to volume, and this can be considered another marker of deformability [8,9,42]. The spherisation index (SI) was lower in alcoholised Wistar rats than in healthy rats (Welch *t*-test: W vs. WA = 55.0 ± 7.5 vs. 40.7 ± 5.8%, $p \leq 0.003$; Figure 5d), indicating a loss of erythrocyte membrane stretching capacity under chronic alcohol exposure. Conversely, in heterozygous rats, SI slightly increased (Welch *t*-test: DH vs. DHA = 46.1 ± 5.7 vs. 52.5 ± 7.5%, $p \leq 0.063$; Figure 5d).

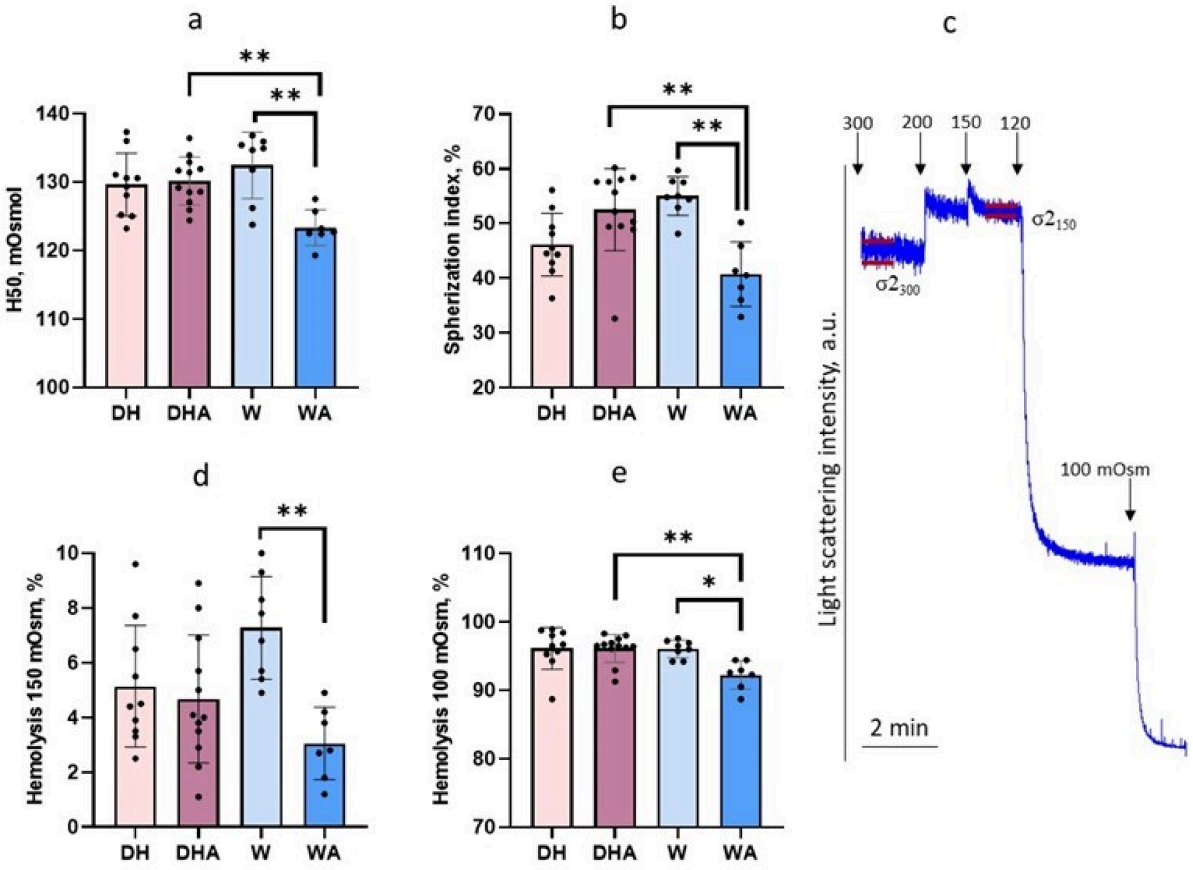


Figure 5. Osmotic resistance of rat erythrocytes. (**a**)—H50-buffer osmolality at which 50% of cells lyse; (**b**)—Spherisation index, calculated as the normalised ratio of light scattering intensity oscillations by erythrocytes at 300 and 150 mOsm; (**c**)—Principle of OFT on the Lasca-TM laser particle analyser; σ2~150~ and σ2~300~—range of light scattering intensity oscillations at 150 and 300 mOsm (highlighted with red lines), used for calculating the erythrocyte spherisation index; (**d**)—% lysis at 150 mOsm (initial stage); (**e**)—% lysis at 100 mOsm (characteristic of process completion and erythrocyte rigidity); The vertical arrows next to the numbers indicate the value of the buffer osmolality at which the recording took place. data are presented as M ± SD; *, $p \leq 0.05$; ** $p \leq 0.01$.

Since osmotic resistance is a composite of the cell's and its membrane's properties (e.g., hydration degree, lipid bilayer cholesterol saturation, and the structural–mechanical properties of cytoskeletal proteins), the osmotic fragility test is a complex characteristic of erythrocyte biophysical properties. Thus, it serves as an indirect assessment of deformability [8,41,43]. OFT results showed that chronic alcohol consumption significantly affected the osmotic fragility of Wistar rat erythrocytes, making them more rigid. This can be interpreted as a typical response to alcohol intoxication [44]. However, alcohol exposure had little effect on the osmotic fragility/rigidity of erythrocytes from genetically modified rats, suggesting that disrupted dopamine transport modifies the biophysical properties of DAT-HET rat erythrocyte membranes, leading to altered sensitivity to hypo-osmotic stress.

2.3. Evaluation of Rat Erythrocytes by Flow Cytometry

Two standard tests that characterise cell viability were used to evaluate the effects of alcohol exposure on erythrocyte cytology. The annexin test was used to evaluate the extent of disruption to lipid asymmetry. The calcein test is a sensitive, fluorescent method of assessing cell viability based on intracellular esterase activity. Calcein-AM can easily enter cells, where esterases cleave the acetoxymethyl (AM) group and release the free calcein molecule, which becomes a highly fluorescent dye. Viability is assessed by mea-

suring calcein fluorescence intensity, which is proportional to the number of live cells and their esterase activity. This test is used to rapidly assess the viability of anucleate human erythrocytes, as it reflects complex disturbances to the enzymes that ensure cell metabolism [45].

The proportion of erythrocytes presenting phosphatidylserine in the DAT-HET rat group was comparable to that in healthy Wistar rats ($p \leq 0.067$; Figure 6a), but the two groups differed substantially in their response to chronic alcohol consumption. Alcohol exposure caused an increase in the externalisation of phosphatidylserine on the outer membrane leaflet of Wistar rat erythrocytes (Figure 6a), which is an expected reaction to the toxic effects of alcohol [46]. For heterozygous rats, the cytological response to alcohol exposure was weak (Welch *t*-test DH vs. DHA: 0.93 ± 0.24 vs. 1.15 ± 0.32%, $p \leq 0.081$).

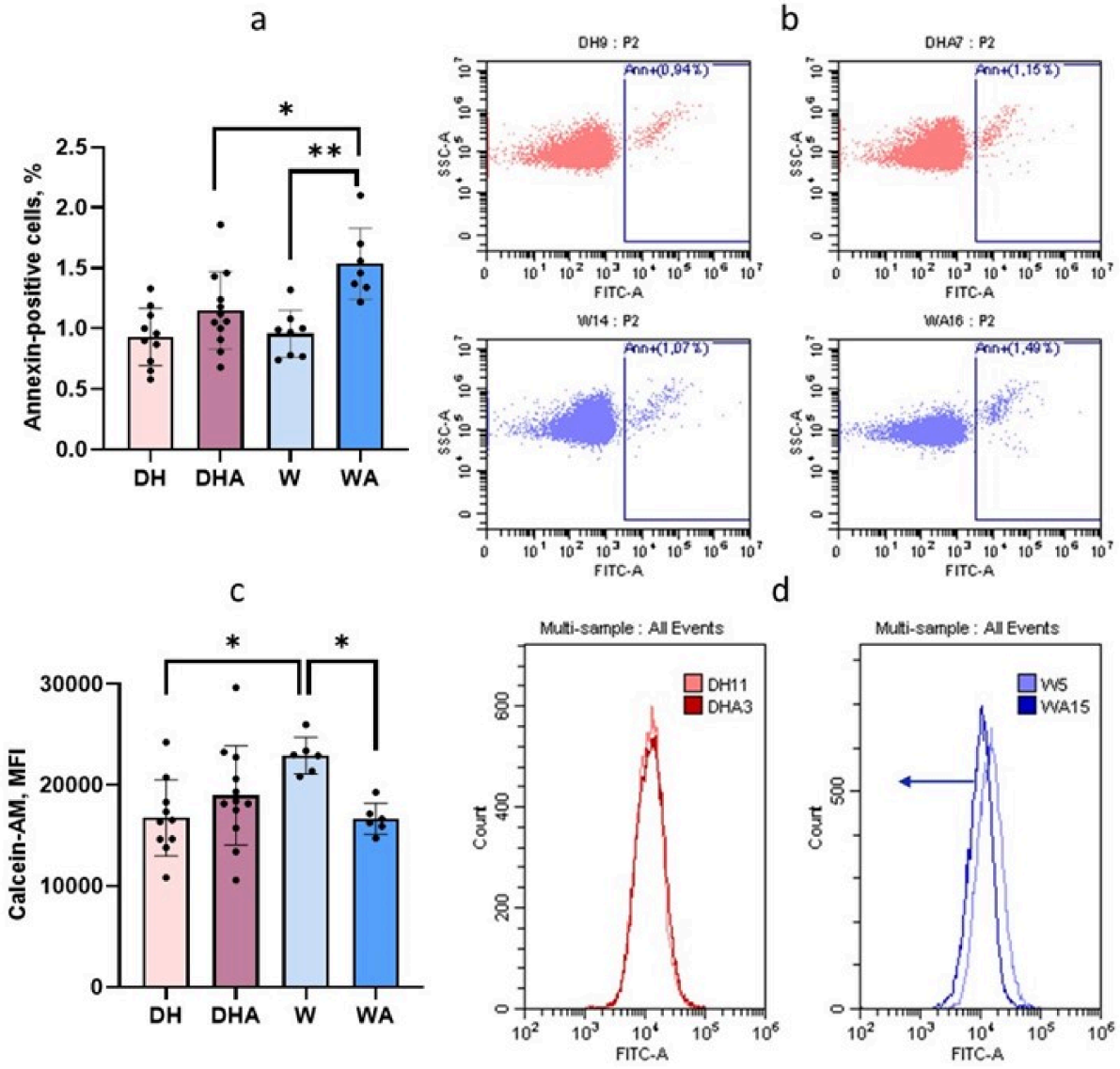


Figure 6. Results of testing the cytological state of rat erythrocytes. (**a**)—Proportion of annexin-positive erythrocytes; (**b**)—Intracellular esterase activity, calcein test; (**c**)—Representative dot-plots and examples of gating erythrocytes to detect annexin-positive cells are shown for erythrocyte samples from all rat groups; DAT-HET rats—pink, Wistar rats—blue; (**d**)—Overlaid histogram showing a shift in calcein fluorescence towards lower values (blue arrow) in an alcohol-consuming rat, WA15, compared to a control Wistar rat, W5. Data are presented as M ± SD; *, $p \leq 0.05$; **, $p \leq 0.01$.

Baseline intracellular esterase activity was lower in DAT-HET rats than in Wistar rats (Figure 6c) at 76% of the Wistar level. The higher intracellular esterase activity in Wistar rat erythrocytes may be associated with a higher reticulocyte level, particularly the young reticulocyte fraction (see Figure 4a), which has a higher metabolic rate than mature cells [47].

Chronic alcohol consumption negatively affected intracellular esterase activity in Wistar rats (Figure 6c). By comparing the decrease in esterase activity with data on annexin-positive erythrocytes, we can observe a typical cellular response to adverse exposure: an increase in the proportion of erythrocytes with disrupted lipid asymmetry (a 1.8-fold increase in annexin-positive cells) and a decrease in overall viability (a 1.4-fold decrease in the fluorescence intensity of calcein-stained erythrocytes).

In heterozygous rats, chronic alcohol consumption did not significantly affect intracellular esterase activity ($p \leq 0.72$). In DAT-HET rats, the reduced rate of intracellular esterase activity, moderate level of annexin-positive cells, and lower production of young reticulocytes are consistent with the hypothesis that erythrocyte turnover is lower in these rats. Unfortunately, this hypothesis cannot be substantiated with direct data on erythrocyte lifespan in genetically modified rats.

In summary, the following conclusions can be drawn from the obtained results:

- Partial knockout of the dopamine transporter gene affects haematopoiesis to a greater extent on the leukocyte component, while the intensity of erythropoiesis in heterozygous rats is approximately equal to the intensity of erythropoiesis in Wistar rats.
- In Wistar rats, chronic alcohol exposure leads to a decrease in erythrocyte count, increased osmotic rigidity (a marker of impaired deformability), and increased signs of apoptotic erythrocyte death (an increased percentage of annexin-positive cells and decreased intracellular esterase activity). This results in a state of anaemia, which is exacerbated by both numerical and functional disturbances to the erythrocytes.
- In DAT-HET rats, which initially differ from Wistar rats in several erythrocyte parameters (MCV, MCH, and esterase activity), chronic alcohol consumption did not lead to statistically significant changes in most of the studied parameters. This may indicate that partial DAT knockout modifies the response of the erythropoietic system and erythrocyte membranes to ethanol-induced stress.

3. Discussion

This study demonstrates, for the first time, that partial knockout of the dopamine transporter gene (DAT-HET) in rats creates a specific baseline haematological profile and fundamentally alters the response of erythrocytes to chronic ethanol exposure. Intact DAT-HET rats exhibited changes in leukocyte production (leukopenia) across major subpopulations (LYM, GRAN, and MID), as well as moderate thrombocytopenia and increased erythrocyte volume (relative macrocytosis).

The erythrogram parameters of Wistar rats corresponded to the reference range for healthy adult rats aged 6–47 weeks [48]. Compared to Wistar rats, the erythroid lineage in DAT-HET rats was characterised by a slight decrease in erythrocyte count and higher cell volume (Figure 3b), with normal haemoglobin and reticulocyte levels. No references to the erythrocyte haematological profile in DAT-KO or DAT-HET rats were found in the literature. However, based on haematogram results in humans with schizophrenia, it can be hypothesised that moderate macrocytosis is one of the signs of dopaminergic signalling disruption [49]. Even a mild degree of anaemia, observed in patients with Alzheimer's disease, may be associated with an increased risk of dementia and cognitive impairment. In Parkinson's disease, the degree of reduction in erythrocyte, haemoglobin, and haematocrit levels depends on the disease stage, with disturbances worsening as

the disease progresses [50]. Thus, even heterozygous DAT inactivation leads to significant restructuring of haematopoiesis, confirming the sensitivity of the blood system to dopaminergic signalling.

Anaemia is a common haematological disorder associated with chronic alcohol consumption. The underlying pathophysiology involves bone marrow suppression, nutrient deficiencies (e.g., folate and iron), and the direct toxic effects of alcohol metabolites on haematopoietic cells [1,51].

In Wistar rats, chronic ethanol consumption over a period of six months induced a pattern of alcohol dependence characterised by anaemia (reduced red blood cell and haemoglobin levels), macrocytosis (increased mean corpuscular volume), a reduced absolute reticulocyte count, increased osmotic rigidity of erythrocytes (decreased H50 and spherisation index), an increased proportion of annexin-positive cells, and decreased intracellular esterase activity. These changes are consistent with the existing literature on the toxic effects of ethanol on erythropoiesis and erythrocyte membranes [1,44]. In humans, elevated MCV is considered a characteristic sign of excessive alcohol consumption and can be observed even in the absence of overt anaemia [1]. Despite the lack of a clear classification for anaemia in rats, we observed the main haematological signs of alcohol-induced haematopoietic disturbance in Wistar rats: anaemia, increased MCH levels, and decreased haematocrit (Figure 5e). In contrast to Wistar rats, chronic alcohol exposure did not lead to statistically significant changes in most erythrocyte parameters studied in DAT-HET rats: osmotic resistance, reticulocyte production, the proportion of annexin-positive cells, and esterase activity remained at control levels. The only exception was a decrease in granulocyte levels, which may indicate preserved sensitivity of the myeloid lineage to ethanol, even in the context of altered dopaminergic signalling.

In Wistar rats, long-term alcohol consumption altered erythrocyte cytological parameters: the level of annexin-positive cells increased significantly, and intracellular esterase activity decreased (Figure 6). Phosphatidylserine-presenting erythrocytes are procoagulant factors and sources of pro-inflammatory and pro-oxidant mediators that exacerbate endothelial dysfunction and inflammation [52]. Moreover, the increased proportion of annexin-positive erythrocytes in alcoholism suggests their enhanced elimination, exacerbating anaemia. The cytological disturbances to erythrocyte membranes were accompanied by changes in membrane biophysical parameters. The functional state of erythrocytes was assessed using the osmotic stress test (OFT), which indirectly indicates deformability disturbances. Chronic alcohol intoxication in Wistar rats caused erythrocyte membrane damage, making them rigid. The haemolysis induced by low buffer osmolality did not reach the values observed in healthy rats (Figure 5e). Erythrocytes represent a heterogeneous population of cells of different ages. The duration of alcoholisation was six months, which, in Wistar rats, is almost three times the lifespan of erythrocytes in circulation after their release from the bone marrow (approximately 45–50 days). Taking this into account, along with the unidirectional changes in osmotic test parameters, it can be concluded that chronic alcohol exposure modifies the biophysical properties of erythrocyte membranes throughout the entire population of Wistar rats. However, the OFT results showed that erythrocyte membranes in heterozygous rats subjected to chronic alcohol exposure did not undergo significant changes affecting their osmotic stability.

The ‘insensitivity’ of erythrocytes to the damaging effects of ethanol can be explained by several mechanisms. Firstly, alterations in dopaminergic signalling may modify the bone marrow microenvironment and the haematopoietic stem cell (HSC) niche, thereby affecting the maturation of erythroid precursors and the properties of erythrocytes released into the blood [24,26]. Secondly, dopamine may influence the lipid composition of membranes and the organisation of the cytoskeleton, which are key factors in deformability and

osmotic resistance. Since erythrocyte cytoskeletal proteins (spectrin and protein 4.1) have homologues in neural tissue that are regulated by dopamine [17,18], it can be hypothesised that genetically determined disruption to dopamine reuptake leads to adaptive changes in membrane properties that increase resistance to ethanol-induced stress.

Dopamine acts as both a neurotransmitter and a cytokine-like signalling molecule for bone marrow cells. Dopamine produced by sympathetic nerves directly controls the behaviour of haematopoietic stem cells (HSCs) via D2-subfamily dopamine receptors. Mechanistically, dopamine controls the expression of lymphocyte-specific protein tyrosine kinase, which in turn regulates MAPK-mediated signalling triggered by stem cell factor in HSCs [24,35]. DAT activity regulates dopaminergic signalling in the immune cell microenvironment, thereby modulating peripheral immunity [35]. It has been demonstrated that single pharmacological doses of haloperidol (a dopamine receptor antagonist) administered to mice result in a reduction in the absolute number of lymphocytes in the peripheral blood and spleen, inducing a negative delayed-type hypersensitivity reaction triggered by BCG vaccination [53].

The observed reduction in circulating immune cell counts in Wistar rats during chronic alcohol consumption (see Figure 1) is consistent with the existing literature. Long-term alcohol consumption is known to alter both innate and adaptive immunity in humans and animals [54]. In individuals with alcohol-use disorder, decreased lymphocyte levels have been observed [54,55], as well as increased production of pro-inflammatory cytokines by monocytes [56]. Similar reactions were observed in mice given 20% alcohol for six months: the development of lymphopenia was accompanied by a redistribution of T-cell subpopulations towards the proliferation of memory cells at the expense of the proliferation of naïve T cells [57]; enhanced NF-κB activation; and increased circulating IL-6 and TNF-α levels in response to lipopolysaccharide stimulation [58]. As with Wistar rats, a trend towards a decrease in the absolute granulocyte count during long-term alcoholisation was observed in DAT-HET rats (Welch t-test, $p \leq 0.026$). In humans with chronic alcoholism, a decrease in granulocyte levels (neutrophils and monocytes/macrophages) has been observed and is attributed to profound disturbances in precursor maturation in the bone marrow [1]. A study by [35] (2023) found that genetic deletion of DAT leads to an enhanced baseline inflammatory phenotype in circulating peripheral myeloid cells. Considering the differences identified in innate (LYM levels) and adaptive (GRAN and MID levels) immunity in DAT-HET rats at baseline and in response to chronic alcoholisation, it can be concluded that an increased alcohol intake exacerbates disturbances in the peripheral immune system of these rats.

A previous study [44] on the functional state of erythrocytes in people with alcohol dependence allows for a comparative analysis of blood cell state during chronic alcohol consumption in Wistar rats and humans. Similar to Wistar rats, the direction and scale of development in patients with alcoholism were altered, but not in DAT-HET rats (Figure 7). Notably, increased osmotic stiffness of erythrocytes is a defining feature of chronic alcohol consumption. In both patients and Wistar rats, haemolysis induced by a hypoosmotic buffer during alcoholisation was incomplete, reflecting impaired erythrocyte deformability. We previously demonstrated in patients that impaired deformability leads to microrheological disturbances; erythrocytes in patients with alcohol dependence exhibited altered flow rates through microchannels [44]. Regarding brain structures, impaired erythrocyte function (rigid membranes, decreased deformability) contributes to increased blood viscosity, impaired capillary blood flow, and neuronal damage [59]. These results confirm the representativeness of the model used to study haematological diseases associated with chronic alcoholism, allowing us to consider the Wistar rat to be a relevant model.

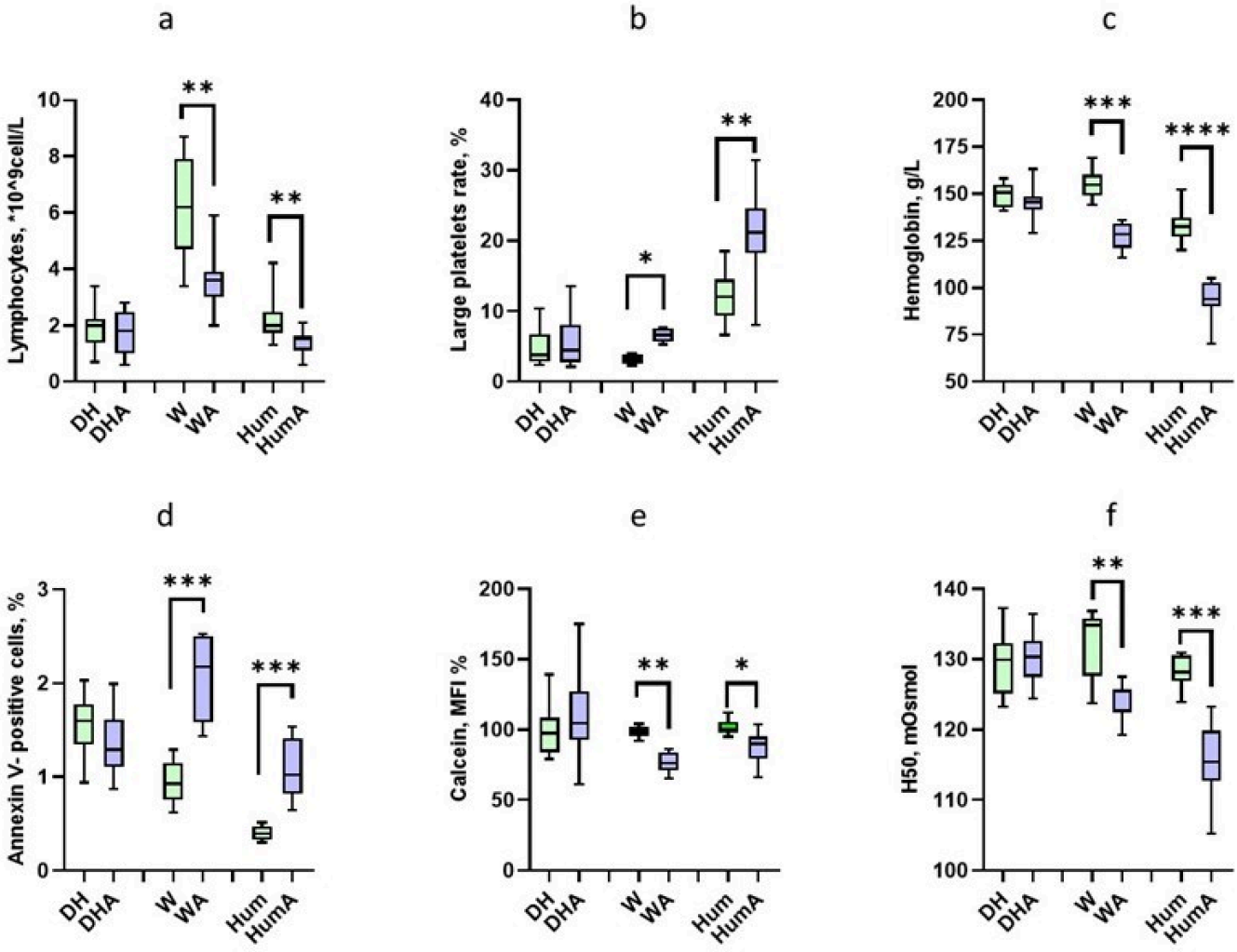


Figure 7. Blood cell response to chronic alcohol consumption in humans and two genetic lines of rats: changes in parameters in humans and Wistar rats exhibited a unidirectional vector: (**a**)—lymphocyte levels decreased; (**b**)—on the background of decreased platelet count, the proportion of large platelets increased; (**c**)—anaemia developed (decreased haemoglobin level); (**d**)—disruption of membrane lipid asymmetry occurred, increasing the level of externally exposed phosphatidylserine; (**e**)—overall erythrocyte viability decreased as assessed by intracellular esterase activity; (**f**)—erythrocyte deformation characteristics were significantly impaired (H50, osmotic resistance parameter). In heterozygous rats, changes in parameters upon alcohol exposure were weakly expressed. Welch *t*-test statistics for all groups: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

In this pilot study, we demonstrated that partial knockout of the dopamine transporter gene (DAT-HET) in rats produces (1) a distinct haematological profile at baseline and (2) significantly alters the erythrocyte response to chronic ethanol exposure. Partial knockout of the dopamine transporter gene (resulting in approximately 50% reduction in functional DAT protein) produces significant alterations in circulating blood cell populations. Compared to Wistar rats, DAT-HET animals demonstrate: profound leukopenia, affecting all major leukocyte subpopulations; moderate thrombocytopenia with increased platelet distribution width (PDW); relative erythrocyte macrocytosis (elevated MCV) with reduced erythrocyte count but normal haemoglobin levels; and reduced intracellular esterase activity (approximately 76% of Wistar levels). These findings establish that even heterozygous disruption of dopamine transporter function significantly impacts haematopoiesis, particularly affecting the leukocyte compartment.

Chronic ethanol exposure produces expected haematological damage in Wistar rats that mirrors human alcohol-use disorder. The six-month alcoholisation protocol (10% ethanol with free choice) induced a pattern consistent with human alcoholism: development of anaemia (reduced RBC and haemoglobin); suppressed erythropoiesis (decreased absolute reticulocyte count); increased erythrocyte osmotic rigidity (reduced H50 and spherisation index); enhanced eryptosis (increased annexin-positive cells); reduced cell viability (decreased esterase activity); lymphocytopenia; and granulocytopenia. This validates the Wistar rat model as representative for studying alcohol-induced haematological disorders in humans.

DAT-HET rats demonstrate resistance to ethanol-induced erythrocyte damage. The most significant finding is that chronic alcohol consumption did not produce any statistically significant changes in the erythrocyte parameters studied in DAT-HET rats: erythrocyte count, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) remained at baseline levels; reticulocyte production showed no significant suppression with a trend toward increased young reticulocytes; osmotic resistance parameters (H50, spherisation index, % haemolysis) remained unchanged; annexin positivity and esterase activity were not significantly altered by ethanol exposure. This suggests that partial DAT knockout fundamentally alters the response of the erythropoietic system and erythrocyte membranes to ethanol-induced stress.

The marked contrast between Wistar rats (which develop alcohol-induced anaemia and erythrocyte dysfunction similar to humans) and DAT-HET rats (which remain largely unaffected) demonstrates that dopaminergic signalling plays a previously unrecognised role in determining erythrocyte sensitivity to alcohol toxicity. These findings open new avenues for understanding the intersection of dopaminergic dysfunction, haematopoiesis, and alcohol-use disorder, with potential implications for managing haematological complications in patients with neuropsychiatric conditions.

4. Materials and Methods

4.1. Reagents and Working Buffers

Calcein-AM (C-AM) was purchased from Molecular Probes (Eugene, OR, USA), Thiazole orange was purchased from Merck (KGaA, Darmstadt, Germany), and buffer components (HEPES, NaCl, KCl, $MgCl_2$, D-glucose, EGTA, $CaCl_2$) were purchased from Sigma-Aldrich (Darmstadt, Germany). Annexin-FITC was obtained from Moleculab (Moscow, Russia). HEPES buffer was contained in mM: NaCl, 140; HEPES, 10; KCl, 2; $MgCl_2$, 2.5; D-glucose, 5; EGTA, 2; and pH 7.4 (pH-meter Mettler Toledo, Columbus, OH, USA). The osmolality of the buffer (300 mOsm/kg H_2O) was controlled by the cryoscopic Semi-Micro osmometer K-7400S (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany).

4.2. Groups of Animals

This work was initiated as part of a study investigating the behavioural characteristics of DAT-HET model rats and their use in the study of dopamine system dysfunction. The DAT-KO transgenic rat model was obtained by editing the genome of Wistar rats (the zinc-finger nuclease editing technique) [21]. Rats with heterozygous inactivation of the DAT gene (DAT-HET) were obtained by crossing homozygous knockout rats (DAT-KO) with Wistar Han rats, resulting in a 50% reduction in restriction sites for the functional dopamine transporter protein [60], which allows comparison of heterozygous rats from both genetic lines. The DAT-KO transgenic rat model was obtained by editing the genome of Wistar rats (the zinc-finger nuclease editing technique), while DAT-HET rats were obtained by crossing DAT-KO and Wistar rats, which allows comparison of heterozygous rats from

both genetic lines. Approval was obtained from the Institute's Bioethics Committee for conducting behavioural experiments (approval date: 29 February 2024, protocol 2-7/2024) and for conducting animal blood tests (approval date: 28 March 2024, protocol 1-2/2024). The work was carried out in accordance with the Declaration of Helsinki.

Two groups of male rats were used in the study: Wistar (W) and DAT-HET (DAT+/−). The chronic alcoholisation regimen lasted six months, during which the rats had continuous access to a 10% ethanol solution and water. This protocol, which was described previously, aims to evaluate alcohol craving in a context of free choice between water and ethanol. Control groups of rats received only water. All animals received a standard diet. The following groups were formed: Wistar control (W, 9 animals/group); Wistar rats receiving alcohol (WA, 7 animals/group); DAT-HET control (DH, 10 animals/group); and DH rats receiving alcohol (DHA, 12 animals/group). Rats were housed two per cage. The amount of ethanol consumed per cage per week was determined and normalised to the animal's body weight per day, calculated as 100% ethanol. No differences in average alcohol consumption were found between the DHA and WA groups ($p \leq 0.537$), providing a basis for comparing the degree of alcoholisation between the groups.

4.3. RBC Preparation

Blood was obtained from rats by decapitation after induction of anaesthesia with 400 mg/kg of chloral hydrate administered intramuscularly. Blood was collected in tubes containing 50 IU of heparin per ml, and all analyses were performed on the same day. A complete blood count was performed using a Medonic M20 haematology analyser (Boule Medical AB, Stockholm, Sweden) in paediatric mode to prevent microclot formation during direct sample measurement.

4.4. Analysis of Rat RBCs by Flow Cytometry

Viability, phosphatidylserine release to the outer surface, and reticulopoiesis activity were studied using standard flow cytometry methods on a CytoFlex flow cytometer (Beckman Coulter, Indianapolis, IN, USA). The erythrocyte gate was established based on preliminary tests involving cells that had been washed free of other blood cells. For annexin and calcein tests, a concentration of 10^6 cells/mL in the appropriate buffer was used. The annexin test detects disruptions in membrane lipid asymmetry. Annexin V-FITC was added to the erythrocyte suspension at a final concentration of 0.1 μg/mL, after which the sample was stained for 15 min at room temperature. Overall erythrocyte viability was assessed by intracellular esterase activity (calcein test). Rat RBCs were incubated with C-AM (0.5 μM for 40 min at 37 °C). For reticulocyte analysis, 5 μL of whole blood in 500 μL of HEPES buffer was stained with thiazole orange (TO) to achieve a final concentration of 0.5 μg/mL. The samples were incubated for 20 min at room temperature. The stained samples were not washed, but immediately transferred for analysis on the CytoFlex flow cytometer, using the 'FITC' channel for all data collection. For the analysis of annexin and calcein staining results, 20,000 events were collected, whereas 100,000 events were collected for reticulocyte analysis. CytExpert 2.3 software (Beckman-Coulter, Brea, CA, USA) was used to collect statistical data.

4.5. Evaluation of Rat RBC Deformability by Laser Diffraction

To assess deformability, an osmotic fragility test (OFT) was performed using a "LaSca-TM" laser particle analyser (BioMedSystem, St. Petersburg, Russia), employing the small-angle light scattering method in the 0–12° range. All experiments were performed in a thermostated cuvette at 37 °C, as previously described [40]. The following parameters were assessed: H50 (the osmolality of the buffer at which 50% of cells lyse); osmotic heterogeneity of the erythrocyte population, which was assessed by the difference in osmolality

at which 10% and 90% lysis occurs, H10–H90, mOsm); and % haemolysis at 150 mOsm (the initial stage of haemolysis after osmotic swelling) and at 100 mOsm (completion of haemolysis for normal erythrocytes). To assess membrane stretching capacity, the spherisation index (SI = $100\sigma_{2150}/\sigma_{2300} \times 100\%$) was used, where σ_{2150} and σ_{2300} are the ranges of light scattering intensity (LSI) oscillations at 150 and 300 mOsm, respectively (adapted from [42]). Erythrocyte spherisation corresponded to a decrease in the LSI oscillation level at 150 mOsm compared to discocytic erythrocytes in their physiological state at 300 mOsm. Thus, the spherisation index (SI) represents the normalised amplitude of light scattering intensity fluctuations (oscillations) generated by erythrocytes in the buffer. Non-uniform light scattering is created by the biconcave discoid shape of the cells. Accordingly, the closer the shape is to spherical, the smaller the difference in light scattering between face and profile views and the smaller the oscillation range. SI is proportional to cell shape asymmetry and can be used to distinguish between normal discocytic and spherical erythrocyte morphology.

4.6. Statistical Analysis

Statistical analyses were performed using Microsoft Excel 2016 (Microsoft Corporation) and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Flow cytometry data were analysed using the proprietary CytExpert software (version 2.3, CytoFLEX, BC). OFT data were analysed using the Lasca-TM laser particle analyser software (original software LaSca_32, v.1498, BioMedSystems Ltd., Saint Petersburg, Russia). Statistically significant differences were determined using analysis of variance (ANOVA) with Dunn's post hoc test for multiple comparisons, or an unpaired Student's *t*-test with Welch's correction, to assess trends in the pilot study setting. The points within the rectangular histograms represent the raw, unprocessed data obtained from each sample (rat) for each parameter. Results are presented as mean ± SD. A p-value ≤ 0.05 was considered statistically significant.

5. Limitations and Future Perspectives

The main limitations of the study are due to its pilot nature and small sample size. The limited statistical power, particularly for subgroup analyses, is a consequence of the small group sizes (n = 7–12). To detect the effect of alcohol, less-stringent statistical formulations were used to show trends, but not differences. As the work is descriptive, it cannot present data on key parameters such as dopamine concentrations in plasma/bone marrow, the expression of dopamine receptors on haematopoietic cells, the analysis of membrane lipid composition, or the state of cytoskeletal proteins (band 3 protein, spectrin, and protein 4.1), which could explain the observed 'resistance' of erythrocytes in heterozygous rats to chronic alcohol exposure. These limitations provide scope for further research, which could focus on:

- Assessing changes in the membrane (lipid composition, cholesterol/phospholipid ratio, and post-translational modifications of cytoskeletal proteins) that underlie the altered response to ethanol in DAT-HET rats;
- Analysing the expression and function of dopamine receptors (especially the D2 subfamily) on haematopoietic stem cells and mature erythrocytes in Wistar and DAT-HET rats;
- Studying the microrheological properties of erythrocytes (microfluidic analysis using biomimetic chips). Another approach to testing the influence of dopaminergic signalling could involve the experimental reproduction of the phenotype observed in DAT-HET rats by using pharmacological manipulation with dopamine receptor antagonists/agonists in Wistar rats.

Author Contributions: E.S.—conceptualisation, conducting blood experiments, and manuscript writing; I.A. and E.F.—animal work and monitoring of alcoholisation; P.S.—breeding and genotyping of the knockout rat line; E.S., I.A. and E.F. analysed the data; E.S., P.S. and I.M. interpreted the results of the experiments; S.G. and I.M.—administration and provision of resources. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the State Assignment of the Ministry of Science and Higher Education of the Russian Federation, Project No. 075-00264-26-00 to E.S., I.A., E.F., S.G., and I.M.; breeding and genotyping of the knockout rat line were performed with financial support from Saint Petersburg State University grant GZ_F_2025-2, No. 148723091, to P.S.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The animal study protocol was approved by the Institutional Ethics Committee of the IEPhB RAS (Protocol No. 1–2 dated 31 January 2024 and Protocol No. 2–7 dated 29 February 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: All the results obtained in this study are presented in the article. The article does not contain any data suitable for archiving in internet databases.

Acknowledgments: The authors thank the Core Facilities Center of the Sechenov Institute of Evolutionary Physiology and Biochemistry for the opportunity to use the CytoFLEX flow cytometer and the cryoscopic Semi-Micro osmometer K-7400S. The authors would like to thank BioMedSystems, Ltd., for the chance to use the LaSca-TM particle analysers. All the results obtained in this study are presented in the article. The article does not contain any data suitable for archiving in internet databases.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DAT	Dopamine transporter
DAT-KO	Dopamine transporter knockout
DAT-HET	Heterozygous dopamine transporter knockout rats (DAT+/− or HET)
DH	DAT-HET, for figures
DHA	DAT-HET rats that consumed alcohol; for figures
W	Wistar rats
WA	Wistar rats that consumed alcohol, for Figures
H50	The osmolality of the buffer at which 50% of cells lyse
H10	The osmolality of the buffer at which 10% of cells lyse
H90	The osmolality of the buffer at which 90% of cells lyse
SI	Spherisation Index
LYM	Lymphocytes
GRAN	Granulocytes, primarily neutrophils
MID	Monocytes, eosinophils, basophils, and their precursors
RBC	Red blood cells
Hum	Human
HumA	Humans who chronically consume alcohol, alcoholics

References

1. Ballard, H.S. The hematological complications of alcoholism. *Alcohol Health Res. World* **1997**, *21*, 42–52.
2. Beaugé, F.; Niel, E.; Hispard, E.; Perrotin, R.; Thepot, V.; Boynard, M.; Nalpas, B. Red blood cell deformability and alcohol dependence in humans. *Alcohol Alcohol.* **1994**, *29*, 59–63. [CrossRef]
3. Bulle, S.; Reddy, V.D.; Padmavathi, P.; Maturu, P.; Puvvada, P.K.; Nallanchakravarthula, V. Association between alcohol-induced erythrocyte membrane alterations and hemolysis in chronic alcoholics. *J. Clin. Biochem. Nutr.* **2017**, *60*, 63–69. [CrossRef]

4. Parmahamsa, M.; Reddy, K.R.; Varadacharyulu, N. Changes in composition and properties of erythrocyte membrane in chronic alcoholics. *Alcohol Alcohol.* **2004**, *39*, 110–112. [CrossRef]
5. Clemens, M.R.; Kessler, W.; Schied, H.W.; Schupmann, A.; Waller, H.D. Plasma and red cell lipids in alcoholics with macrocytosis. *Clin. Chim. Acta* **1986**, *156*, 321–328. [CrossRef] [PubMed]
6. Benedetti, A.; Birarelli, A.M.; Brunelli, E.; Curatola, G.; Ferretti, G.; Del Prete, U.; Jezequel, A.M.; Orlandi, F. Modification of lipid composition of erythrocyte membranes in chronic alcoholism. *Pharmacol. Res. Commun.* **1987**, *19*, 651–662. [CrossRef]
7. Boşgelmez, I.; Güvendik, G.; Dilbaz, N.; Esen, M. Differential Expression of Erythrocyte Proteins in Patients with Alcohol Use Disorder. *Int. J. Mol. Sci.* **2025**, *26*, 8199. [CrossRef] [PubMed]
8. Huisjes, R.; Bogdanova, A.; van Solinge, W.W.; Schiffelers, R.M.; Kaestner, L.; van Wijk, R. Squeezing for Life—Properties of Red Blood Cell Deformability. *Front. Physiol.* **2018**, *9*, 656. [CrossRef]
9. Cabrales, P. Effects of erythrocyte flexibility on microvascular perfusion and oxygenation during acute anemia. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *293*, H1206–H1215. [CrossRef] [PubMed]
10. Li, J.; Lykotrafitis, G.; Dao, M.; Suresh, S. Cytoskeletal dynamics of human erythrocyte. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4937–4942. [CrossRef]
11. Weisel, J.W.; Litvinov, R.I. Red blood cells: The forgotten player in hemostasis and thrombosis. *J. Thromb. Haemost.* **2019**, *17*, 271–282. [CrossRef]
12. Skverchinskaya, E.; Filippova, O.; Gambaryan, S.; Bukatin, A.; Koloskov, A.; Mindukshev, I. Decompensated Liver Cirrhosis Impairs the Deformability of Erythrocytes and Their Ability to Pass Through Microchannels. *Biochem. Suppl. Ser. A Membr. Cell Biol.* **2026**, *20*, 103–117. [CrossRef]
13. Faria-Pereira, A.; Morais, V.A. Synapses: The Brain's Energy-Demanding Sites. *Int. J. Mol. Sci.* **2022**, *23*, 3627. [CrossRef] [PubMed]
14. Clementi, M. Changes In Erythrocyte Deformability In Alzheimer's Disease: A Role For Nitric Oxide Synthase. *Alzheimer's Dement.* **2009**, *5*, e12. [CrossRef]
15. Ouazia, D.; Levros, L.C., Jr.; Rassart, E.; Desrosiers, R.R. Dopamine down-regulation of protein L-isoaspartyl methyltransferase is dependent on reactive oxygen species in SH-SY5Y cells. *Neuroscience* **2014**, *267*, 263–276. [CrossRef]
16. Pretorius, E.; Swanepoel, A.C.; Buys, A.V.; Vermeulen, N.; Duim, W.; Kell, D.B. Eryptosis as a marker of Parkinson's disease. *Aging* **2014**, *6*, 788–819. [CrossRef]
17. Cuadrado-Tejedor, M.; Sesma, M.T.; Giménez-Amaya, J.M.; Ortiz, L. Changes in cytoskeletal gene expression linked to MPTP-treatment in Mice. *Neurobiol. Dis.* **2005**, *20*, 666–672. [CrossRef]
18. Walensky, L.D.; Blackshaw, S.; Liao, D.; Watkins, C.C.; Weier, H.U.; Parra, M.; Huganir, R.L.; Conboy, J.G.; Mohandas, N.; Snyder, S.H. A novel neuron-enriched homolog of the erythrocyte membrane cytoskeletal protein 4.1. *J. Neurosci.* **1999**, *19*, 6457–6467. [CrossRef]
19. Vaughan, R.A.; Foster, J.D. Mechanisms of dopamine transporter regulation in normal and disease states. *Trends Pharmacol. Sci.* **2013**, *34*, 489–496. [CrossRef]
20. Klein, M.O.; Battagello, D.S.; Cardoso, A.R.; Hauser, D.N.; Bittencourt, J.C.; Correa, R.G. Dopamine: Functions, Signaling, and Association with Neurological Diseases. *Cell. Mol. Neurobiol.* **2019**, *39*, 31–59. [CrossRef]
21. Sukhanov, I.; Leo, D.; Tur, M.A.; Belozertseva, I.V.; Savchenko, A.; Gainetdinov, R.R. Dopamine transporter knockout rats as the new preclinical model of hyper- and hypodopaminergic disorders. *Bechterev Rev. Psychiatry Med. Psychol.* **2019**, *4*, 84–85. [CrossRef]
22. Cinque, S.; Zoratto, F.; Poleggi, A.; Leo, D.; Cerniglia, L.; Cimino, S.; Tambelli, R.; Alleva, E.; Gainetdinov, R.R.; Laviola, G.; et al. Behavioral Phenotyping of Dopamine Transporter Knockout Rats: Compulsive Traits, Motor Stereotypies, and Anhedonia. *Front. Psychiatry* **2018**, *9*, 43. [CrossRef] [PubMed]
23. Gainetdinov, A.R.; Fesenko, Z.S.; Khismatullina, Z.R. Behavioural Changes in Heterozygous Rats by Gene Knockout of the Dopamine Transporter (DAT). *J. Biomed.* **2020**, *16*, 82–88. [CrossRef]
24. Liu, Y.; Chen, Q.; Jeong, H.W.; Han, D.; Fabian, J.; Drexler, H.C.A.; Stehling, M.; Schöler, H.R.; Adams, R.H. Dopamine signaling regulates hematopoietic stem and progenitor cell function. *Blood* **2021**, *138*, 2051–2065. [CrossRef]
25. Cosentino, M.; Marino, F.; Maestroni, G.J. Sympathoadrenergic modulation of hematopoiesis: A review of available evidence and of therapeutic perspectives. *Front. Cell. Neurosci.* **2015**, *9*, 302. [CrossRef]
26. Spiegel, A.; Kalinkovich, A.; Shivtiel, S.; Kollet, O.; Lapidot, T. Stem cell regulation via dynamic interactions of the nervous and immune systems with the microenvironment. *Cell Stem Cell* **2008**, *3*, 484–492. [CrossRef]
27. Yang, J.X.; Li, Y.Y.; Deng, Z.H.; Li, T.J.; Bai, K.; Yu, Y.M.; Ma, J.; Chen, Q.; Liu, Y. D2-type dopamine receptors are required for LPS-induced acute hematopoiesis. *FEBS Lett.* **2025**, *599*, 3318–3331. [CrossRef]
28. Bolan, E.A.; Kivell, B.; Jaligam, V.; Oz, M.; Jayanthi, L.D.; Han, Y.; Sen, N.; Urizar, E.; Gomes, I.; Devi, L.A.; et al. D2 receptors regulate dopamine transporter function via an extracellular signal-regulated kinases 1 and 2-dependent and phosphoinositide 3 kinase-independent mechanism. *Mol. Pharmacol.* **2007**, *71*, 1222–1232. [CrossRef] [PubMed]

29. Bliziotes, M.; Gunness, M.; Eshleman, A.; Wiren, K. The role of dopamine and serotonin in regulating bone mass and strength: Studies on dopamine and serotonin transporter null mice. *J. Musculoskelet. Neuronal Interact.* **2002**, *2*, 291–295.
30. Yang, W.; Singla, R.; Maheshwari, O.; Fontaine, C.J.; Gil-Mohapel, J. Alcohol Use Disorder: Neurobiology and Therapeutics. *Biomedicines* **2022**, *10*, 1192. [CrossRef]
31. Lauretani, F.; Giallauria, F.; Testa, C.; Zinni, C.; Lorenzi, B.; Zucchini, I.; Salvi, M.; Napoli, R.; Maggio, M.G. Dopamine Pharmacodynamics: New Insights. *Int. J. Mol. Sci.* **2024**, *25*, 5293. [CrossRef] [PubMed]
32. Heinz, A. Dopaminergic dysfunction in alcoholism and schizophrenia--psychopathological and behavioral correlates. *Eur. Psychiatry* **2002**, *17*, 9–16. [CrossRef]
33. Chang, J.-S.; Huang, H.-Z.; Yuan, M.; Zhou, Y.; Liu, D.; Zhan, K.-B.; Zhu, L.-Q. Alcohol addiction and Alzheimer's disease: A molecular collision course. *Transl. Psychiatry* **2025**, *15*, 410. [CrossRef] [PubMed]
34. Luderer, M.; Ramos Quiroga, J.A.; Faraone, S.V.; Zhang James, Y.; Reif, A. Alcohol use disorders and ADHD. *Neurosci. Biobehav. Rev.* **2021**, *128*, 648–660. [CrossRef]
35. Gopinath, A.; Mackie, P.M.; Phan, L.T.; Mirabel, R.; Smith, A.R.; Miller, E.; Franks, S.; Syed, O.; Riaz, T.; Law, B.K.; et al. Who Knew? Dopamine Transporter Activity Is Critical in Innate and Adaptive Immune Responses. *Cells* **2023**, *12*, 269. [CrossRef]
36. Wang, J.Y.; Ye, S.; Zhong, H. The role of bone marrow microenvironment in platelet production and their implications for the treatment of thrombocytopenic diseases. *Hematology* **2017**, *22*, 630–639. [CrossRef]
37. Samuel, D.; Bhat, A.N.; Prabhu, V.M. Platelet Indices as Predictive Markers of Prognosis in Critically Ill Patients: A Prospective Study. *Indian J. Crit. Care Med.* **2020**, *24*, 817–822. [CrossRef]
38. Zhang, J.; Liu, Y.; Han, X.; Mei, Y.; Yang, J.; Zhang, Z.J.; Lu, X.; Ji, P. Rats provide a superior model of human stress erythropoiesis. *Exp. Hematol.* **2019**, *78*, 21–34.e3. [CrossRef]
39. Derelanko, M.J. Determination of erythrocyte life span in F-344, Wistar, and Sprague-Dawley rats using a modification of the [3H]diisopropylfluorophosphate ([3H]DFP) method. *Fundam. Appl. Toxicol.* **1987**, *9*, 271–276.
40. Gerda, B.A.; Skverchinskaya, E.A.; Andreeva, A.Y.; Volkova, A.A.; Gambaryan, S.; Mindukshev, I.V. A Comparative Analysis of Erythrocyte Osmotic Fragility across Vertebrate Taxa. *J. Evol. Biochem. Physiol.* **2024**, *60*, 1363–1384. [CrossRef]
41. Sudnitsyna, J.; Skverchinskaya, E.; Dobrylko, I.; Nikitina, E.; Gambaryan, S.; Mindukshev, I. Microvesicle Formation Induced by Oxidative Stress in Human Erythrocytes. *Antioxidants* **2020**, *9*, 929. [CrossRef]
42. Skverchinskaya, E.; Levdarovich, N.; Ivanov, A.; Mindukshev, I.; Bukatin, A. Anticancer Drugs Paclitaxel, Carboplatin, Doxorubicin, and Cyclophosphamide Alter the Biophysical Characteristics of Red Blood Cells, In Vitro. *Biology* **2023**, *12*, 230. [CrossRef]
43. Orbach, A.; Zelig, O.; Yedgar, S.; Barshtein, G. Biophysical and Biochemical Markers of Red Blood Cell Fragility. *Transfus. Med. Hemother.* **2017**, *44*, 183–187. [CrossRef]
44. Walski, T.; Chludzińska, L.; Komorowska, M.; Witkiewicz, W. Individual Osmotic Fragility Distribution: A New Parameter for Determination of the Osmotic Properties of Human Red Blood Cells. *BioMed Res. Int.* **2014**, *2014*, 162102. [CrossRef]
45. Sohma, H.; Ohkawa, H.; Hashimoto, E.; Sakai, R.; Saito, T. Ethanol-induced augmentation of annexin IV expression in rat C6 glioma and human A549 adenocarcinoma cells. *Alcohol. Clin. Exp. Res.* **2002**, *26*, 44s–48s. [CrossRef]
46. Diana Matei, D.; Ghișe, A.; Huțu, I.; Cireșan, A.; Dumitrescu, E. The laboratory rat physiological parameters. *Rom. J. Vet. Sci.* **2025**, *58*, 320–328. [CrossRef]
47. Ransing, R.; Kamble, A.; Kulkarni, R.; Khairkar, P.; Patil, S.; Pevekar, K.; Misha, K.; Gangane, N. Common Hematological Disorders in Patients with Schizophrenia: In-Patient Case—Control Study. *I.P. Pavlov. Russ. Med. Biol. Her.* **2022**, *30*, 193–202. [CrossRef]
48. Taneva, S.G.; Todinova, S.; Andreeva, T. Morphometric and Nanomechanical Screening of Peripheral Blood Cells with Atomic Force Microscopy for Label-Free Assessment of Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis. *Int. J. Mol. Sci.* **2023**, *24*, 14296. [CrossRef] [PubMed]
49. Scheiner, B.; Semmler, G.; Maurer, F.; Schwabl, P.; Bucsics, T.A.; Paternostro, R.; Bauer, D.; Simbrunner, B.; Trauner, M.; Mandorfer, M.; et al. Prevalence of and risk factors for anaemia in patients with advanced chronic liver disease. *Liver Int.* **2020**, *40*, 194–204. [CrossRef] [PubMed]
50. Boas, L.V.; Faustino, V.; Lima, R.; Miranda, J.M.; Minas, G.; Fernandes, C.S.V.; Catarino, S.O. Assessment of the Deformability and Velocity of Healthy and Artificially Impaired Red Blood Cells in Narrow Polydimethylsiloxane (PDMS) Microchannels. *Micromachines* **2018**, *9*, 384. [CrossRef]
51. Kim, K.H. Effects of Antipsychotic Drugs on Cellular lmmunity in Mice. *Yonsei Med. J.* **1986**, *27*, 59–66. [CrossRef] [PubMed]
52. Barr, T.; Helms, C.; Grant, K.; Messaoudi, I. Opposing effects of alcohol on the immune system. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2016**, *65*, 242–251. [CrossRef] [PubMed]
53. Mili, F.; Flanders, W.D.; Boring, J.R.; Annest, J.L.; DeStefano, F. The associations of alcohol drinking and drinking cessation to measures of the immune system in middle-aged men. *Alcohol. Clin. Exp. Res.* **1992**, *16*, 688–694. [CrossRef]

54. Pang, M.; Bala, S.; Kodys, K.; Catalano, D.; Szabo, G. Inhibition of TLR8- and TLR4-induced Type I IFN induction by alcohol is different from its effects on inflammatory cytokine production in monocytes. *BMC Immunol.* **2011**, *12*, 55. [CrossRef] [PubMed]
55. Zhang, H.; Meadows, G.G. Chronic alcohol consumption in mice increases the proportion of peripheral memory T cells by homeostatic proliferation. *J. Leukoc. Biol.* **2005**, *78*, 1070–1080. [CrossRef]
56. Marasliogl u, M.; Oppermann, E.; Blattner, C.; Weber, R.; Henrich, D.; Jobin, C.; Schleucher, E.; Marzi, I.; Lehnert, M. Chronic ethanol feeding modulates inflammatory mediators, activation of nuclear factor-κB, and responsiveness to endotoxin in murine Kupffer cells and circulating leukocytes. *Mediat. Inflamm.* **2014**, *2014*, 808695. [CrossRef]
57. Lee, H.; Heo, J.; Lee, I.H.; Kim, Y.D.; Nam, H.S. Association between blood viscosity and early neurological deterioration in lacunar infarction. *Front. Neurol.* **2022**, *13*, 979073. [CrossRef]
58. Leo, D.; Sukhanov, I.; Zoratto, F.; Illiano, P.; Caffino, L.; Sanna, F.; Messa, G.; Emanuele, M.; Esposito, A.; Dorofeikova, M.; et al. Pronounced Hyperactivity, Cognitive Dysfunctions, and BDNF Dysregulation in Dopamine Transporter Knock-out Rats. *J. Neurosci.* **2018**, *38*, 1959–1972. [CrossRef]
59. Antonova, I.V.; Kutcher, E.O.; Filatova, E.V.; Veraksa, A.E.; Morina, I.Y.; Zavialov, V.A.; Egorov, A.Y. Changes in Behavioral Characteristics and Tyrosine Hydroxylase Levels in the Nucleus Accumbens of the Brain of DAT-HET Rats during Free Alcoholization. *J. Evol. Biochem. Physiol.* **2023**, *59*, 586–595. [CrossRef]
60. Mikhailova, D.M.; Skverchinskaya, E.; Sudnitsyna, J.; Butov, K.R.; Koltsova, E.M.; Mindukshev, I.V.; Gambaryan, S. Hematin- and Hemin-Induced Spherization and Hemolysis of Human Erythrocytes Are Independent of Extracellular Calcium Concentration. *Cells* **2024**, *13*, 554. [CrossRef]

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