HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

MSc Molecular Biomedicine

"ALEXANDER FLEMING"
Biomedical Sciences Research Center

INTERNATIONAL TRANS - INSTITUTIONAL POSTGRADUATE PROGRAM

"MOLECULAR BIOMEDICINE: MECHANISMS OF DISEASE, MOLECULAR AND CELLULAR THERAPIES AND BIOINNOVATION"

Medical School, National and Kapodistrian University of Athens,
in collaboration with B.S.R.C. "Alexander Fleming"

MSc Thesis

Effects of early life adversity on the reward anticipation system of the rat

Dimitra Fousfouka

Supervisor

Prof. Antonios Stamatakis

Professor, Faculty of Nursing, National and Kapodistrian University of Athens, Greece

ATHENS, 2023

HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

MSc Molecular Biomedicine

"ALEXANDER FLEMING"
Biomedical Sciences Research Center

Three Member Evaluation Committee

Antonios Stamatakis, Professor, Faculty of Nursing, National and Kapodistrian University of Athens, Greece

Christos Consoulas, Associate Professor, Laboratory of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, Greece

Efthimios Skoulakis, Researcher A' (Research Professor), Division Leader, Neuroscience Division B.S.R.C. "Alexander Fleming", Vari, Greece

Acknowledgments

The present work was conducted at the Faculty of Nursing, School of Health Sciences of the National and Kapodistrian University of Athens, at Prof. Antonios Stamatakis laboratory from September 2022 until September 2023.

I would like to express my deepest gratitude and appreciation to all those who have contributed to the successful completion of my master thesis. Firstly, I would like to thank my supervisor, Prof. Antonios Stamatakis for the opportunity he gave me to join his research group, for his valuable support and guidance and for sharing his knowledge and passion for research. I would also like to thank my lovely labmates and especially Ermis Ryakiotakis, PhD candidate, for the time he invested to train me and for his continuous help and support. Additionally, I am grateful to Dr. Athanasios Stergiopoulos, Post-doc Researcher and Lydia Pavlidi, Michaela Kourla, PhD candidates of Prof. Stamataki's group, for our collaboration, making an enjoyable working environment.

Moreover, I would like to thank Dr. Christos Consoulas and Dr. Efthimios Skoulakis for participating in the evaluation of this project.

Finally, I would like to express my gratitude to my family and close friends and especially to my best friend Christina who was always by my side.

Part of the present work has been included in the research article:

1. Ryakiotakis E., **Fousfouka D.** and Stamatakis A. (2023) Maternal neglect alters reward-anticipatory behavior, social status stability, and reward circuit activation in adult male rats. *Frontiers in Neuroscience.* 17:1201345. doi: 10.3389/fnins.2023.120134

Abstract

It is well-documented that early life experiences have long term effects in both humans and animals, influencing adult brain function and behavior. Specifically, early life stress induces behavioral changes in adult rats, like anhedonia, aggressive behavior, and social avoidance, as well as biochemical alterations including dopamine (DA) release disruption in many brain areas. Prefrontal cortex (PFC) and nucleus accumbens (nAC), two key components of reward system, are among the most affected. Understanding the biological mechanisms underlying the effects of early life stress in later life remains a great challenge. Earlier studies from our laboratory indicated that an animal model of adverse early life experiences, in the form of denial of expected reward (DER), simulating maternal neglect can affect the dopaminergic as well as the reward system.

Using the DER model, we investigated the long-term impact of an adverse neonatal experience on the behavioral and biochemical components of the reward system. For this purpose, we evaluated the anticipatory behavior and the motivational response by performing reward learning tasks as well as the levels of dopamine receptors D1R in ventral PFC and nAC of adult male DER and control animals both under basal conditions and following the reward learning tasks.

Our results indicate that the DER animals showed defects both in context-reward and cue-reward associations. Moreover, they exhibited modified levels of dopamine receptors D1R in both vPFC and nAC compared to controls. The knowledge of the behavioral effects of early life stress as well as the underlying molecular neurobiological mechanisms could feed-in to mechanism-based targeted therapies to prevent/treat psychological disorders related to child/juvenile trauma.

Contents

Abbreviations

AC – Adenylate cyclase

ACTH – Adrenocorticotropic hormone

ADHD – Attention deficit hyperactivity disorder

B – Basal

CNS – Central nervous system

CoP – Contextual-dependent learning Phase

CuP – Cue-dependent learning Phase

CRH – Corticosterone

CTR – Control animals

D1R – type-1 like Dopamine Receptor

D2R – type-2 like Dopamine Receptor

DA – Dopamine

DAT – Dopamine transporter

DER – Animals Denied the Expected Reward

cAMP – Cyclic adenosine monophosphate

EH – Early handling

ELS – Early life stress

Exp – Experimental

FA – Food anticipation

GEE – Generalized estimated equations

GLM – Generalized linear model

GR – Glucocorticoid receptors

HPA – Hypothalamic-Pituitary–Adrenal axis

MD – Maternal deprivation

mPFC – Medial prefrontal cortex

MS – Maternal separation

nAC – Nucleus accumbens

PFC – Prefrontal cortex

PKA – Protein Kinase A

PLC – Phospholipase C

PND – Postnatal day

PTSD – Post-traumatic stress disorder

SEM – Standard error of the mean

TH – Tyrosine hydroxylase

vlPFC – Ventral lateral prefrontal cortex

VMAT – Vescicular monoamine transporter

vmPFC – Ventromedial prefrontal cortex

vPFC – Ventral prefrontal cortex

VS – Vetral striatum

VTA – Ventral tegmental area

1. Introduction

1.1. Early Life Experiences

It is well-documented that early life experiences have long term effects in both humans and animals, influencing adult brain function and development, as well as behavior, effects persisting even in adulthood [1]. The relationship between mother and infant plays a pivotal role in the development of the child. It is well known that a warm, intimate, and stable relationship between the mother (or permanent caregiver) and the infant and young child, as well as a "good enough" maternal care are prerequisites for the development of normal adult behavior, is essential for normal development and mental health. Disrupting the mother-infant relationship can lead to long-term physiological and behavioral consequences for the offspring [2]. Abandonment or abuse by the mother during the critical developmental period can lead to psychopathology [3, 4, 5].

Early life stress (ELS), often known as early life adversity and childhood trauma, is a broad term that refers to the exposure to chronic or severe stressful life events during childhood and adolescence [6]. ELS consists of prenatal, perinatal, and postnatal stress [7]. ELS can affect the development of the hypothalamic-pituitary–adrenal (HPA) axis, an essential part of the organism's stress response system, causing cellular and molecular alterations in the developing brain that can result in neurobehavioral changes later in life. Furthermore, ELS can affect brain function, and neurobehavior [8].

A large number of data obtained in humans as well as in animal studies have demonstrated that early life events which interfere with or disrupt mother infant interactions, alter the development and the activity of the mesolimbic and mesocortical dopaminergic systems [9, 10, 11, 12]. Specifically early life stress (ELS) in the form of parental neglect/abuse/maltreatment/loss has been associated with a wide range of mental disorders including impaired social cognition and emotion recognition combined with increased risk factor for psychiatric disorders such as schizophrenia, bipolar disorder, borderline personality disorder, major depression, and post-traumatic stress disorder [13].

Maternal behavior is undeniably a determinant factor of adult behavior [14]. Adults who have been exposed to ELS, manifest reward system deficiencies such as lower behavioral drive when faced with an unexpected increase of monetary reward [15, 16] as well as attenuated ventral striatum (VS)

response during reward reception and formation of positive social relationships [17, 18, 19]. The developing brain is highly responsive to many influences from the mother [20].

Decades of research in the field of Developmental Psychobiology provide experimental evidence for the significance of the relationship between mother and her offspring for the development of adult behaviors, an idea that had been originally proposed by psychologists such as Sigmund Freud, Melanie Klein, Mark Spitz and John Bowlby. A stable mother-infant interaction and a "good enough" maternal care are prerequisites for the development of normal adult behavior, while abandonment or abuse by the mother during the critical developmental period can lead to psychopathology [3, 4]. Early experiences have been shown to affect cognitive abilities in adulthood [21]. Adverse early experiences are known to increase the risk for emotional psychopathology, such as depression, in the etiopathology of which the serotonergic system is intimately involved. Early experiences have been considered as one of the most important factors in determining future adaptability to the demands of the environment, as well as predisposition to mental and physical diseases [3,4,5].

1.2. Experimental models of adverse early life experiences

Animal models play a fundamental role in understudying the neurobiological mechanisms of early life experiences. Different animal models of ELS have been developed throughout the years. In most animal models of early life experiences maternal care was the factor which was manipulated [14].

As outlined above, the parent-child relationship during the early postnatal development period is of critical importance for the survival of the offspring. Therefore, the quality of this relationship has been the focus of both clinical and preclinical research in the last decades. Because so far there is little experimental data on the psychobiology of child neglect, the need for the development of rodent and primate models of early life manipulations appeared crucial. In mammals, the mother is the principal caregiver, providing the offspring with both nutritional resources for its survival and behavioral stimulation that will enable the appropriate social skills to develop [22]. In this framework, experimental paradigms involving manipulation of the mother-infant relationship have been used extensively to investigate the nature of the developmental impact of these interactions. However, these manipulations are often disruptive of the development of the offspring. The

outcomes mostly depend on a modified development of the HPA axis and therefore several animal models developed to study the mechanisms underlying neonatal plasticity of the HPA system.

1.2.1. Early Handling (EH)

Early handling (EH) is a mild early life stressful experience that affects stress related brain systems leading to increased adaptation in future challenges. The handling procedure itself involves removing the pups from the nest and placing them in a different cage. Infants are separated from their mother either as individual pups or as litter. This brief separation usually lasts 15 min and then the pups return to the litter cage. This procedure is repeated daily for different time frames during the lactation period, until weaning [23].

It has been shown that EH evokes changes in body temperature, short-term transient increases in thyroid hormones, hippocampal serotonin (5-HT) activity, hippocampal cAMP formation, protein kinase A, mRNA and protein levels of cAMP – responsive transcription factors and hippocampal glucocorticoid receptor mRNA and protein levels [24, 25]. It has also been found that EH causes long-term changes in brain metabolic capacity [26]. Moreover, EH was found to stimulate active maternal care [27, 28].

The long-term effects of EH include adaptive responses of the stress axis following exposure to stressors in adulthood. More specifically, handled rats exhibited decreased emotionality in novel environments and a hyporesponsive HPA axis in the form of increased but temporally contained secretion of adrenal glucocorticoids in response to stress as well as increased cognitive performance [29, 30, 31]. These data showed that early life experiences can modify the development of adaptive responses to stress in later life. Handled rats have lower corticosterone secretion in response to stress and a faster hormone drop back to basal levels after the termination of stress, compared to non-handled rats [23].

EH has also been widely used to investigate the impact of early life experiences on brain plasticity. As mentioned above, early handling is linked to lifelong increase in GR density in the hippocampus of rats but may also accelerate the maturation rate of hippocampus neuroanatomically, thereby enhancing learning and memory. This was supported by data showing enhanced contextual fear memory at a very early age [32]. Glucocorticoid hypersecretion during and after learning/conditioning may interfere with consolidation of memory [33], thus, neonatal handling may

facilitate contextual fear conditioning by moderating the level of corticosterone secretion during postconditioning memory consolidation. Neonatal handling reduces the HPA response to stress, an effect that persists over the lifespan.

Concerning cognition and behavior, handling has been reported to have the following effects in adult rats: Enhances two-way active avoidance learning [34], reduces emotional reactivity as assessed by the expression of low anxiety-like behavior and high exploratory behavior, which was accompanied by low secretion of corticosterone in response to stress [35, 36], and reduces susceptibility to helplessness [37]. It has also been reported that EH impairs inhibitory avoidance but enhances object recognition, leading to alterations in learning and memory [38]. It was also found that repeated brief maternal separations can reduce social behavior in adult rats by reducing social investigative interaction and increasing aggressive behavior [39]. Furthermore, handled rats exhibit decreased behavioral inhibition in a novel and potentially harmful situation and increased maternal aggressive behavior [40], casting doubts on the generalization that neonatal stimulation has only beneficial effects on the behavior of adult rats. However, others have demonstrated that handling increases playfulness leading to positive social emotions like joy [41].

In general, it is proposed that brief maternal separations in the form of early handling is beneficial regarding adaptation to environmental challenges later in life. Nevertheless, this conclusion may vary implying a genetic influence and a dissociation of the effects depending on the different behaviors. Additionally, some studies suggest that the behavioral changes observed in adult rats during EH attribute to the mother's increased licking behavior [24, 42]. However, it has also been reported that mothers briefly separated from their pups exhibited no significant changes in maternal behavior, nevertheless their offspring exhibited certain behavioral changes in adulthood similar to those of the handled group [43]. These results indicate increased maternal care cannot be the sole factor accounting for the long-term effects of early handling.

1.2.2. Maternal separation (MS) – Maternal deprivations (MD)

Maternal separation or deprivation is an animal model study of early life adversity, which resembles more the effects of maternal deprivation in human studies on child neglect. It consists of long periods of maternal absence from 1 to 24h in the case of MD, and shorter periods in the case of MS, on one or multiple days, during the neonatal period [44, 45]. These separations seem to lead to

reduction of enzymes related to development, decreases of heart rate, increased sensitivity of HPA axis and a general behavior suppression, similar to those in depression [46]. Rats that have been exposed to MD/MS deal with difficulties in integration and social interaction [47]. Given the fact that MD, especially for prolonged periods, causes not only alterations in the HPA axis, but also in the processing of auditory stimuli, neurochemical systems of the brain, and cognitive function, it has been proposed as model of schizophrenia and depression-like behaviors [48]. Collectively, pups that were exposed to MD or MS during early life, as adults show increased levels of ACTH, GRs and prolonged secretion of corticosterone (CRH) in adulthood [49, 50, 51, 52].

1.2.3. DER model

In our laboratory a novel animal model of early life experience has been developed, which simulates maternal neglect, one of the most common forms of ELS. This model includes a learning component. More specifically, during post-natal days (PNDs) 10–13, before eye opening, rat pups learn a T-maze, using contact with the mother as a positive reinforcer [53]. Specifically, rat pups are trained for 10 trials per day, individually in a T-maze, in which one arm leads to the mother-containing cage. When reaching this cage, the pups are not allowed to be retrieved by their mother since the door remains closed (Denied the Expected Reward, DER). It should be noted that all pups are returned to the cage containing the mother and the whole litter immediately after the end of the daily training, which lasts approximately 10 min. Therefore, the DER pups do receive their mother's care, but with a short delay of approximately 10 min. Interestingly, it was found that DER pups receive increased maternal care compared to the controls (not trained at all in the T-maze) [54]. So, the DER group is initially denied maternal contact, but receives it later with a delay of ~10 min. Additionally, the DER pups during the period they are in the T-maze and particularly throughout the waiting time in front of door leading to the mother-containing cage, are exposed to the odor of the nest and the mother, which is a stimulus that has been shown to dampen ultrasonic vocalizations [55, 56, 57] an indicator of separation distress. As a result, based on the last two points, we hypothesized that the DER experience is of a relatively mild negative valence, but with elements of frustration. Specifically, our model refers to early life experiences that are not such extreme as, for example, maltreatment or abuse, but to behaviors that are mildly adverse, conditions that occur more often among humans. The DER pups learn the T-maze, that is, with training they decrease the time it takes them to find

the mother-containing cage and progressively make more successful trials, that is, they find the entrance of the mother-containing cage. Moreover, pups develop a clear preference for the right arm of the T-maze, that is, the one leading to their mother containing cage, making more turns into it. The improvement in performance with time is not only a reflection of developmental processes of motor maturation, since the performance on day 13 of pups exposed to the T-maze only on PND10 and day 13, was much worse than that of pups trained for 4 days (days 10 through 13) [53].

The T-maze training takes place during PND 10–13, because it is the period before rat pups open their eyes, which occurs between PND 13-14. So, rat pups can only use their olfaction to find the mother cage [54]. Moreover, during PND 10-13, starts prefrontal cortex (PFC) development (Figure 1), [1].

It was also demonstrated that the DER experience affects brain neurochemistry. It leads to brain activation, reflecting the ongoing brain processing of information, and interacts with the ongoing developmental processes, leading to long-term consequences, altering the function of these areas in the adult rat brain. Furthermore, DER experience affects the HPA axis. Notably, it increases GR levels, possibly due to increased maternal care. It also increases the levels of CRH and corticosterone. Moreover, DER adult animals displayed anhedonia, aggression and a hypofunctioning serotonergic system, leading to depression-like behavior. Furthermore, DER pups exhibited decreased DA levels in the PFC persisting into adulthood [54]. They also had fewer glutamatergic neurons and lower neuronal density in the medial orbital and infralimbic cortex accompanied by poor performance in attention set-shifting tasks [58]. These findings suggest that the DER experience can have long-lasting effects on the PFC in general and specifically on its dopaminergic system, possibly affecting its contribution to the reward circuitry function. Based on the above, DER experience could be a good animal model for human situations related to maternal neglect [59].

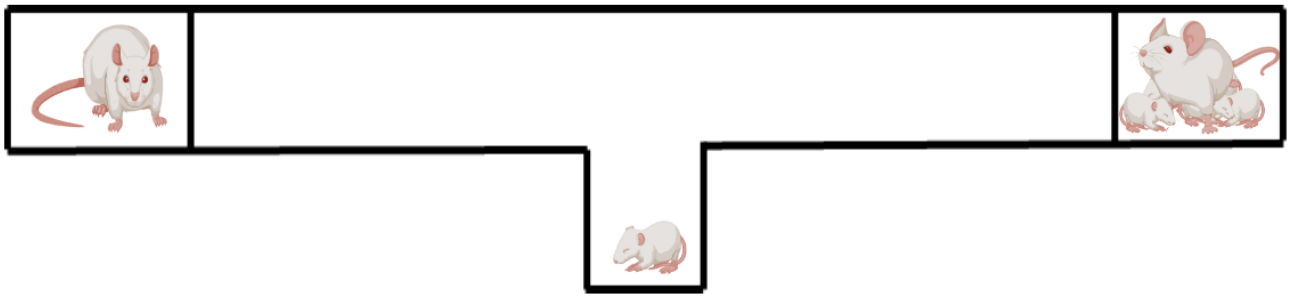

Figure 1. Schematic representation of the DER model in a T-maze.

1.3. Dopaminergic System

More than 50 years have passed since the discovery of the physiological function of a metabolite of the amino acid tyrosine, known as dopamine [61, 62]. Dopamine (DA) is a neurotransmitter in the central nervous system (CNS) that has distinct functions in the adult brain, such as regulation of motor functions and motivation. DA is linked to mobility increases during anticipation and is associated with activation of the reward system. The dopaminergic system plays a pivotal role in a wide range of functions of CNS including conscious control of movements, reward, food, sleep, emotional, reproductive, and maternal behavior, cognitive functions such as attention, learning and memory, decision making, or aggression [63, 64, 65, 66, 67, 68, 69].

1.3.1. Dopaminergic Pathways

DA synthesis occurs in nerve cells of the midbrain, mainly in substantia nigra (SN) and ventral tegmental area (VTA) and is released in the nucleus accumbens (nAC) and the prefrontal cortex (PFC). Neurons producing DA are categorized in four major dopaminergic pathways: the mesolimbic pathway, the mesocortical pathway, the nigrostriatal pathway, and the tuberoinfundibular pathway [70]. The mesocortical pathway transmits DA from the VTA (in the midbrain) to the cortex (PFC) and controls cognitive and executive functions such as working memory, attention, mental flexibility, self-control, and planning. The mesolimbic pathway from VTA to the ventral striatum, including nAC, and hippocampus controls memory, reward, motivation, and addiction. The nigrostriatal pathway starting from the substantia nigra to the striatum (caudate nucleus and putamen) is mainly involved in motor functions. The tuberoinfundibular pathway transmits DA from the hypothalamus to the pituitary and plays a major role in regulation of hormones secretion, for example prolactin [71, 72], (Figure 2).

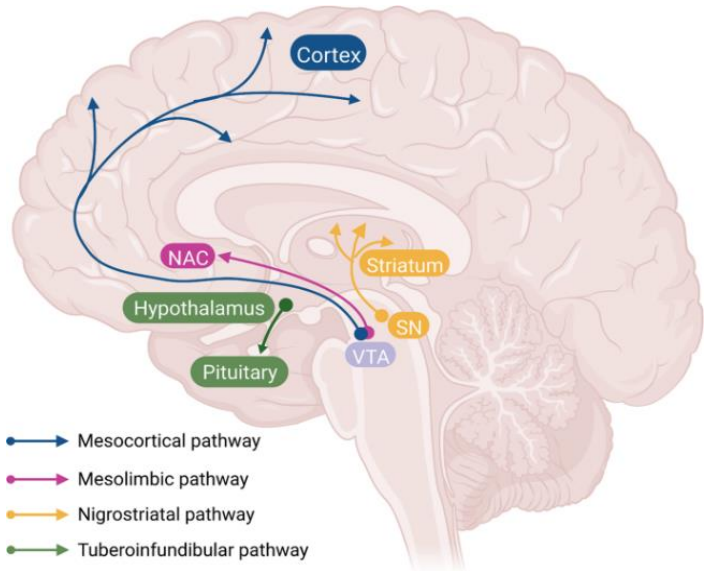

Figure 1. Main dopamine pathways in the brain [73].

1.3.2. Dopaminergic Synapse

The regulation of DA synthesis, metabolism and release in the brain is mostly controlled by three enzymes: Tyrosine Hydroxylase (TH), Monoamine Oxidase (MAO) and Catechol-O-methylTransderase (COMT), and two transporters: DAT and Vescicular Monoamine Transporter (VMAT). DAT is the key regulatory element regarding the reuptake of DA in the presynaptic cell. DA is synthesized by removing a carboxyl group from a molecule of its precursor chemical, L-DOPA. More specifically, L-tyrosine is converted into L-DOPA by the enzyme tyrosine hydroxylase and then L-DOPA is converted into DA by Dopa decarboxylase. After synthesis, DA is transported from the cytosol into synaptic vehicles by VMAT [74, 75].

DA activates dopamine receptors which are coupled to G-proteins. Dopamine receptors are classified into type-1 like (D1R-like) and type-2 like (D2R-like) dopamine receptors. Briefly, D1R causes activation of adenylate cyclase (AC), which increases cAMP and leads to activation of PKA and phosphorylation of DARPP-32 on Thr34 [76]. Moreover, D1Rs activate PLC and thus affect intracellular calcium concentration. D2Rs inhibit the action of AC, and consequently affect neurotransmitter release. They also inhibit tyrosine hydroxylase (TH), the enzyme responsible for dopamine synthesis (Figure 3).

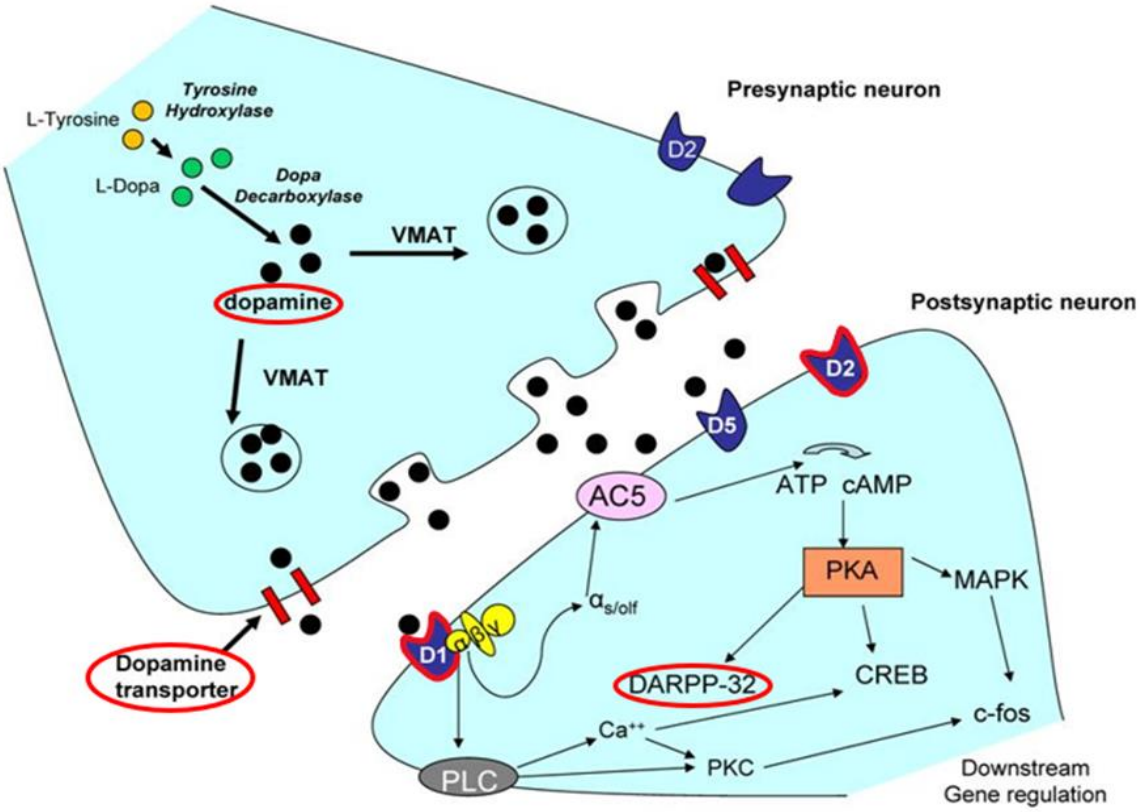

Figure 3. Representation of dopaminergic synapse [77].

1.3.3. Dopamine Receptors

Dopamine receptors are a class of G coupled-proteins. DA receptors have been divided into two major subtypes referred to as D1 and D2, which differ in their coupling to G-proteins, their distribution within the CNS, and their pharmacology. There are also many other receptors that mimic the activity of D1 receptor, called type-1 like (D1R-like) dopamine receptors, including D5 receptor. Furthermore, there are type-2 like (D2R-like) dopamine receptors that mimic the activity of D2 dopamine receptor, consisting of D3 and D4 receptors.

DA binds to type-1 like (D1R-like) and type-2 like (D2R-like) dopamine receptors. D1R-like binding mediates in neurotransmission, while D2R-like receptors inhibit neurotransmission. Dopamine transporter (DAT) is the key regulatory element regarding the removal of DA from the synapse by presynaptic reuptake (Figure 3) [75, 78].

D1R is the most abundant out of the five in the CNS, followed by D2R, then D3R, D5R and D4R. D1R is widely expressed in the brain with the highest levels being found in nigrostriatal, mesolimbic, and mesocortical circuits including nAC, substantia nigra, olfactory bulb, amygdala and frontal lobe [79]. It has been shown that there is decreased expression of dopaminergic receptors under conditions of early life stress. Neural connection between nAC and VTA occurs via two pathways: directly and indirectly. In the direct pathway, nerve cells of nAC that express D1R project to the VTA directly, whereas in the indirect pathway nerve cells that express D2R project to the ventral globus pallidus and then to the VTA. Later studies indicated that the direct pathway is involved in reward-related behaviors [78], while the indirect pathway is implicated in the activation of nerve cells (D2R) that are related to impulsive emotions [80, 81].

1.3.4. Dopaminergic System and ELS

It is well-known that ELS is responsible for many alterations in brain structure and function, which could have long-term effects in adulthood [82, 83] and is associated with a high risk of developing psychiatric disorders [62, 84]. A large number of animal and human studies have revealed that early life experiences which interfere with or disrupt mother–infant interactions, can cause alterations in development and activity of the mesolimbic and mesocortical dopaminergic systems that drive motivated behavior [76, 85, 86, 87, 88].

It has been shown that neonatal handling reduces DA release after stress and leads to sex dimorphic alterations of DA levels and its turnover in different brain regions [89] while it increases D1R density in the nAC [90]. It has also been shown that prolonged MS and isolation of rat pups leads to modified levels of D1R and D2R in the striatum and the PFC, as well as elevated basal and stress-induced DA levels, while decreased DAT levels in the striatum in adulthood [76, 91, 92, 93]. Furthermore, repeated maternal separation results in elevated striatal concentrations of DA and a reduced turnover of this neurotransmitter in the PFC [85]. Moreover, adult offspring of high-licking and grooming mothers exhibit alterations of DA levels in the medial PFC in response to stress [94]. Thus, the dopaminergic system seems to be quite sensitive to early-life manipulations and environmental factors [86, 88, 91, 95].

1.4. Reward System

The reward system links together a number of brain structures that control the ability of feeling pleasure. When the reward system is activated, each individual cell in the circuit relays electrical and chemical signals. The brain regions that are responsible for mediating the physiological and cognitive processing of reward are collectively referred to as mesolimbic or reward system. Reward is a natural physiological process during which the brain associates different stimuli (substances, situations, events, or activities) with a positive or desirable outcome [96].

Reward is crucial for survival. It forces animals to approach stimuli and engage in fitness-enhancing behaviors (eating, drinking, and mating). For the majority of animal species, survival depends on increasing exposure to beneficial stimuli and reducing contact with detrimental ones. Evolution will favor species that are capable of reaping better rewards. For this reason, the brain works to achieve rewards in the most efficient manner possible. However, the brain needs first to identify the reward value of objects for survival (reward cognition), before directing the acquisition of these reward objects through learning, strategy, decision-making, and positive emotions. This central function of the brain is served by sensory discrimination and motor regulation. For this purpose, nature has provided animals with distinct neuronal reward circuits that process all crucial aspects of reward functions. Therefore, reward is a mechanism that evolved to increase the adaptive fitness of animals [97, 98].

Therefore, the reward system is responsible for reward-related cognition, including several psychological components: liking (hedonic reaction to the pleasure of a reward), wanting

(motivation process of incentive salience), and learning (Pavlovian or instrumental responses and cognitive assosiations) (Figure 4). These processes have distinct neural mechanisms and can occur simultaneously during the reward-behavior cycle (Figure 4).

The reward system includes many brain areas e.g. VTA, ventral striatum (nAC and olfactory tubercle), dorsal striatum (caudate nucleus and putamen), substantia nigra (pars compacta and pars reticulata), prefrontal cortex, anterior cingulate cortex, insular cortex, hippocampus, hypothalamus, thalamus, subthalamic nucleus, globus pallidus, ventral pallidum, parabrachial nucleus, amygdala.

As it has already been mentioned, DA plays a fundamental role in a variety of important brain functions such as reward, cognition, motor control, emotion, sexual and maternal behavior, aggression, and pair bonding. The dopaminergic system is regarded as the central neural sensor of rewarding experiences that drives motivated behavior toward desired goals [99, 100].

The dopaminergic pathways mostly involved in reward are the mesolimbic and mesocortical systems. These dopaminergic systems are basic components of the reward circuit underlying many of the above behaviors by providing the necessary drive for learning on one hand and the consummatory element of emotionally laden behaviors such as sexual and maternal behavior [101]. The mesolimbic/mesocortical circuit consists predominantly of specialized neurons that synthesize DA and are located in the VTA which project to brain areas such as the nAC and the PFC [102, 103, 104]. When rewarding stimuli are experienced, the dopaminergic mesolimbic/mesocortical system is activated which causes the release of DA from the VTA to the targeted nuclei. The VTA has two key pathways, the mesolimbic pathway, which projects to limbic (striatal) regions and mediates motivational behaviors and processes, and the mesocortical pathway, which projects to the PFC and controls cognitive processes. There is also a feed-in circuit in the system, since the PFC can modulate the release of DA in the nAC [102, 103]. The ventral striatum, including nAC, is a major substrate involved in reward, mediating the effects of natural and artificial rewards [105, 106]. Moreover, this reward sub-circuit plays a pivotal role in the ‘‘wanting’’ and seeking part of the reward processes [106]. Notably, DA release has been correlated with the sense of reward anticipation and acquisition [107], while drugs such as amphetamine, which prolong DA presence in the synaptic cleft are extremely addictive [108, 109, 110]. DA-DA receptor binding is crucial for the manifestation of reward-driven behaviors [111]. In classical Pavlovian conditioning, cue-reward onset induces phasic DA release from VTA. VTA neuron firing rate on nAC predicts the expected value of an upcoming reward [112]. For example, increases in phasic DA release promote learning association between a

natural reward (unconditioned stimulus) and the environmental cues that predict it (conditioned stimulus) [113].

It is well established that adult humans exposed to ELS manifest reward system deficiencies such as lower behavioral drive when faced with an unexpected increase in monetary reward [114] as well as attenuated ventral striatum (VS) response during reward reception and formation of positive social relationships [115, 116, 117]. Furthermore, a large body of data obtained in humans as well as in animal studies has shown that early-life events which interfere with or disrupt mother–infant interactions can alter the development and activity of the mesolimbic and mesocortical dopaminergic systems that drive motivated behavior [76, 85, 86, 88]. The impact on perception of rewards as well as on goal-oriented behavior is either directly mediated by the downregulation of DA receptor expression or indirectly mediated by the manipulation of VTA function via changes to the HPA axis [118, 119]: For instance, DA release is reduced by CRH secretion into VTA [120, 121]. These results emphasize the significance of early age for the physiological brain and behavioral maturation and suggest that ELS constitutes a determinant factor in the development of psychiatric diseases, including schizophrenia and attention deficit hyperactivity disorder (ADHD), particularly through alterations to the reward system. Indeed, increasing data from both human and animal studies indicate the association of mood disorders and drug addiction with major disruptions within the brain's reward circuit. Furthermore, evidence from human studies suggests that the structural and functional alterations in reward system are linked with reward-related behavioral impairments in mental disorders [122].

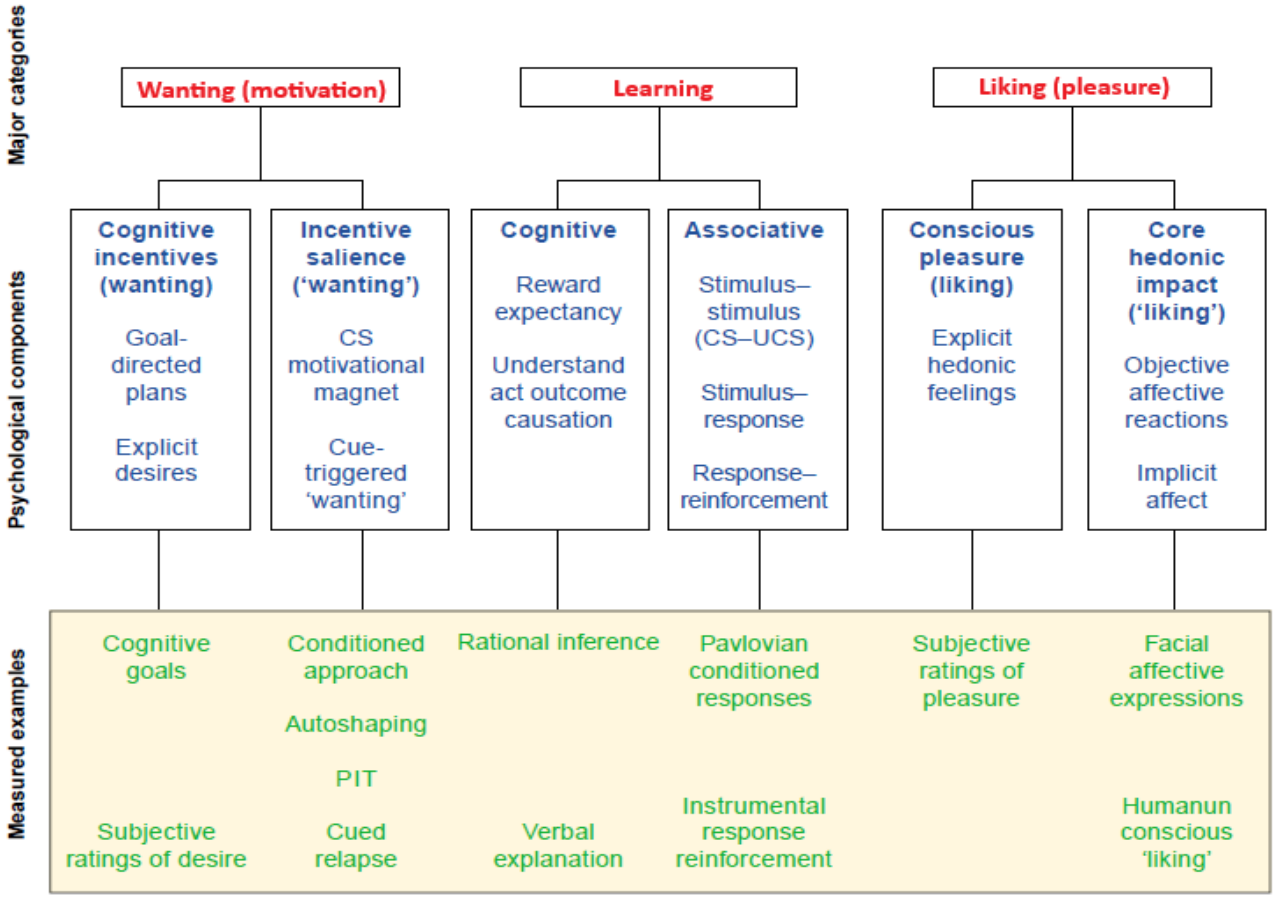

Figure 4. Components of reward system [100].

1.5. Prefrontal cortex – Nucleus accumbens

Anatomically, PFC covers the rostral part of the frontal lobe of the cerebral cortex. It plays a central role in cognitive control functions, including, for example, planning, decision making, working memory, personality expression, moderating social behavior and controlling certain aspects of speech and language [123]. Prefrontal cortex (PFC) development is the longest of any brain area, taking more than two decades to achieve full maturity in humans [124], while in rats its maturation continues into adolescence [125].

The ventral prefrontal cortex (vPFC) contains the ventromedial (vmPFC) and ventrolater (vlPFC) prefrontal cortex. It interconnects with brain regions involved with emotion [126]. Ventral PFC neurons fire during reward collection while inactivating the ventral mPFC delayed the collection of reward [127]. It has been associated with habitual behaviors and emotional regulation, among multiple other functions. Ventral mPFC manipulation has been shown to regulate behavioral inhibition in certain circumstances, such as during fear extinction [123, 124]. Ventromedial

prefrontal cortex (vmPFC) is engaged in reward valuation and integrates different outcome attributes. It evaluates the reward reception, as well as modulates whether the behavior is sufficient to receive the reward [128, 129]. It is also implicated in reversal learning [130].

The lateral ventral prefrontal cortex or ventrolateral prefronal cortex (vlPFC) is part of the vPFC located in the frontal lobes. vlPFC plays a role in inhibiting inappropriate or impulsive behaviors [131]. It is also involved in maintaining and manipulating information in working memory and cognitive functions [132]. Additionally, vlPFC is also implicated in emotion regulation and decision making not driven by emotional reactions. [133].

Nucleus accumbens (nAC) is located in the rostral and ventral forebrain. It is a major component of the ventral striatum that has long been thought to be a key structure involved in mediating motivational and emotional processes. It is part of the reward system and plays an important role in processing rewarding and reinforcing stimuli [134]. nAC is involved in various cognitive, emotional, and psychomotor functions. It serves as an important area for motivation, reward and pleasure, addiction, impulsivity and risk-taking behaviors. It is also involved in the control of survival and reproductive behaviors.

It has been documented that early life adversity affects the development of PCF and nAC. It can disrupt early brain development and reduce the volume and thickness of PFC, leading to loss of prefrontal cognitive abilities [135]. It is well known that ELS alters brain structure with many findings coming from human studies and specifically from children raised in orphanages or humans who experienced other types of ELS. There is strong evidence showing decreased gray and white matter volume and differences in cortical thickness in children that have spent time in institutions. Therefore, these results indicate abnormal PFC and nAC development in institutionalized children. Furthermore, the structural and morphological changes are accompanied by functional and connectivity alterations that in turn have been associated with cognitive and behavioral outcomes in children, adolescents, and adults. These results also affect emotional- and reward- related tasks in children and adolescents [136].

Evidence coming from animal studies indicates that ELS in animals during early postnatal days alters brain development and can lead to dysfunction in neurotransmitter systems, brain connectivity and behavior, that persist until adulthood [137]. Early life adversity can induce an altered biochemical profile responsible for neuronal function in many brain areas, such as alterations in dopamine levels [138]. Prefrontal cortex (PFC) and nucleus accumbens (nAC), two key areas responsible for the behavioral responses in reward learning, are among the most affected brain areas [135]. Moreover,

PFC and other brain regions show altered connectivity in the brain of animals that experienced ELS. Behaviorally, animal models of ELS demonstrate alterations in many tasks that test for anxiety, fear, cognition, memory, learning and social behaviors. Additionally, results coming from animal studies of ELS show structural/functional deficits in PFC as well as in nAC [136].

1.6. Aim of the study

The aim of this study was to explore the impact of adverse early life experiences on naturally evoked motivated behaviors and their underlying reward circuit mediators, namely the vPFC and the nAC. Using a rat model of early life adversity, the DER experience, we investigated the effects of our model on the dopaminergic and reward system. The dopaminergic circuit is an important component of the reward system and appears to be sensitive to early life experiences. Specifically, the purpose of the present study was to investigate the effects of the DER experience on the behavioral components of the reward system, i.e., the impact of DER experience on the expectation of an upcoming reward (reward anticipation) and the motivation to receive a reward (motivational response) including context motivational response and cue evoked motivational response.

According to earlier findings from our laboratory, that the DER experience results in decreased dopamine levels in the PFC both in pups and adult animals [54], we hypothesized that the DER experience can disrupt normal responses to natural rewards [100]. To this end, we investigated the development of anticipatory behavior in a Pavlovian cue-reward association task performed by groups of animals. In addition, based on previous studies that showed dopamine dysfunction in DER animals, we wanted to delve into the long-term changes in dopaminergic system determining in both the vPFC and the nAC of adult rats, the levels of dopamine receptor D1R both before and following reward anticipation training. Even though the protein levels of type - 1 dopamine receptor (D1R) in the PFC and nAC have been documented to be unaffected by basal conditions in adult DER rats [101], no relevant data are currently known regarding tasks under reward anticipation.

To address the establishment of reward anticipatory behavior we used grouped food anticipation (FA) trials to assess how the DER experience can act as a modulator of reward cognition during group learning. It should be noted that ELS is also associated with abnormal social behaviors such as increased aggression and lower social responses in humans. Thus, disruption of normal social behaviors due to ELS can constitute an additional social stressor that could escalate

psychopathological progression [136, 139] and precipitate DER-induced reward system malfunctions.

The behavioral end-points we determined were the total distance moved and the rearing activity in an open field arena across the days of training. General locomotion is a widely used marker to describe conditioned anticipatory behavior robustness. Rearing activity is an exploratory activity that is elevated during anticipation [140]. Additionally, we measured the protein levels of type -1 dopamine receptor (D1R) under basal conditions (no behavioral training) as well as following the final day of food anticipation in vPFC and nAC to explore the effects of DER experience on the dopaminergic circuit following reward anticipation establishment.

2. Materials and Methods

2.1. Animals

Wistar rats used in the following experiments were bred in our colony (EL 25 BIObr 43). Animal living conditions follow the standard guidelines for rat housing: three animals were housed together in a Plexiglas cage with a mesh wire top and had ad libitum access to water and food (food pellets, Kounker-Keramari Bros. and Co., Athens, Greece) in a 12 h light/day cycle (07:00 a.m.−19:00 p.m.) at 22–23^{o}C. The bedding (wood chip) was replaced weekly. Prior to birth, each litter was randomly allocated to the control group or the denial of expected reward (DER) group (see below). Litters having 3–6 males and 6–10 animals in total were used in order to have comparable levels of nest conditions and avoid extreme variations in pup body weight and maternal behavior. To avoid disturbing the litter or the mother, cages were not cleaned, but instead wood chips were added. After weaning (PND 22), all animals were left undisturbed (except for weekly cage cleaning) until the start of the behavioral experiments (PND 80). At weaning, rats were separated from their mothers and housed in the following groups: 3xDER male (DER group) and 3xCTR male (CTR group). Each group was, randomly, assigned to the basal or experimental conditions, and all animals in each cage were littermates: Under basal conditions, animals were not behaviorally tested in adulthood, while under experimental conditions, animals were behaviorally tested in adulthood. All experiments were carried out in agreement with the ethical recommendations of the European Communities Council Directive of 22 September 2010 (2010/63/EU) and approved by our institution's ethical committee (authorization number #291, Athens, 2019). All efforts were made to minimize animal suffering and to reduce the number of animals used.

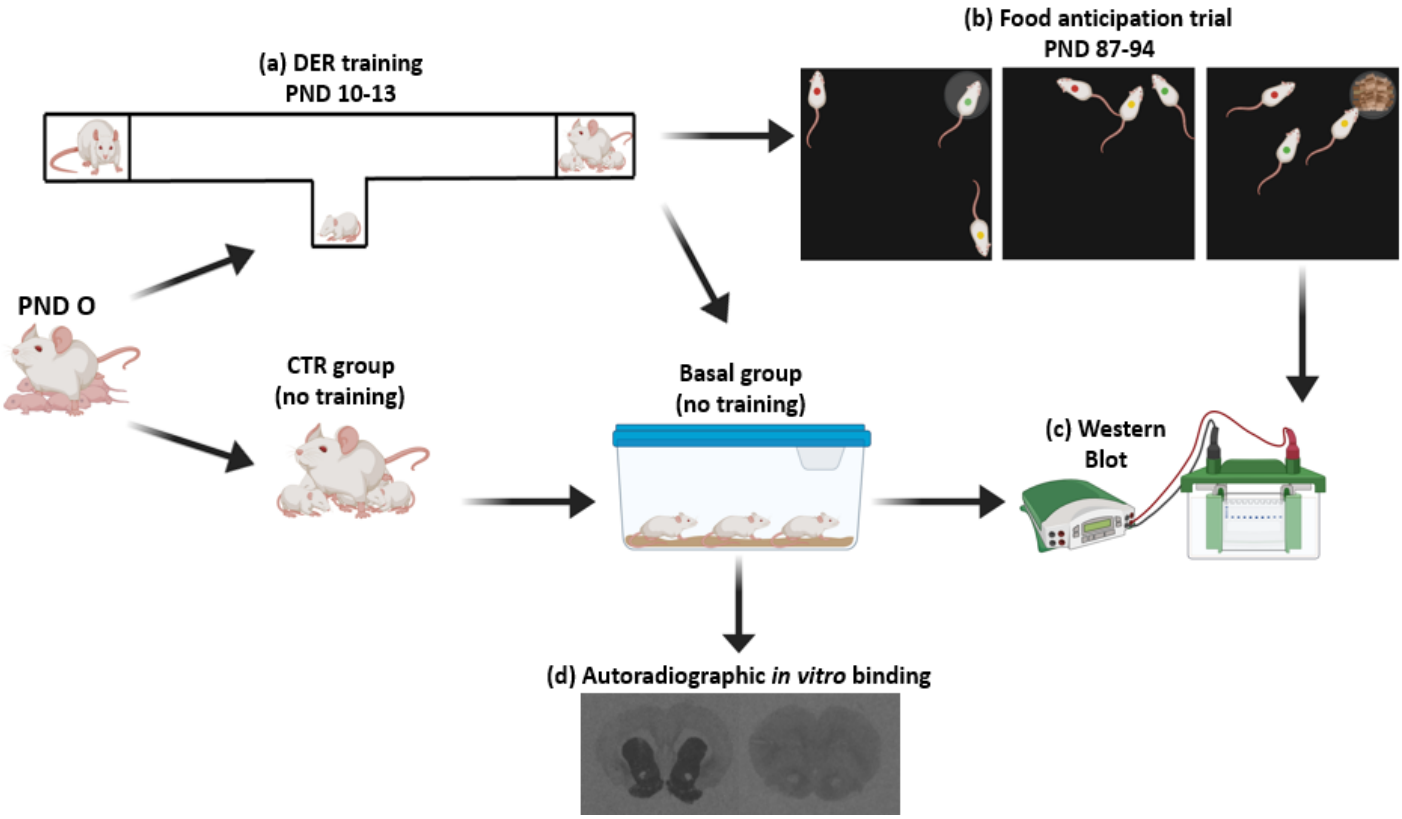

Figure 5. Experimental procedure. (a) PND 10–13: rat pups of the DER group underwent DER training. Animals of the CTR group remained undisturbed. (b) PND 87–94: food anticipation training was carried out for seven consecutive days. Day 0 served as the accommodation trial. Food was removed from the home cage for the rest of FA training. (c) PND 94: animals were killed immediately after the end of day 7 food anticipation training, brain tissues were harvested, and Western blots were performed for D1R protein levels determination. Adult animals (DER and CTR) in the basal group were not exposed to any behavioral testing in adulthood. For these animals, Western blots (d) as well as *in vitro* binding were performed for D1R binding levels determination.

2.2. Behavioral testing

Animals underwent a series of behavioral and biochemical testing summarized in Figure 5.

2.2.1. Neonatal T-maze training

A model of mild early life adversity has been developed in our lab, in which pups are trained in a T-maze while being denied the expected reward of maternal contact (DER). As previously described in detail [21, 53], we used a custom-made T maze where the cage with the mother and litter was placed at the end of one arm of a T-maze, and a cage with a virgin adult female rat was placed at the end of the other arm. Plastic mesh at the gate of each cage prevented physical contact with the animals in either cage. Each rat pup was placed at the center of the T-maze, free to explore for 60secs. If the

pup successfully reached the mother-containing cage, it remained in front of the entrance for 20 sec, and then was returned to the center of the T-maze; otherwise, it was gently pushed to the entrance of the mother-containing cage and left there for 20 sec before returned to the center of the T-maze. This was repeated 10 times per day for 4 days from post-natal day 10 to 13 (PND 10–13). Between pups, the T-maze was cleaned thoroughly with 70% ethanol. To retain the original home cage smell and amplify the olfactory cues, each day some bedding (one handful) was transferred from the home cage to the mother-containing cage in the T-maze. CTR animals did not undergo any training and were left undisturbed with their mother until weaning apart from weekly husbandry procedures (Figure 6).

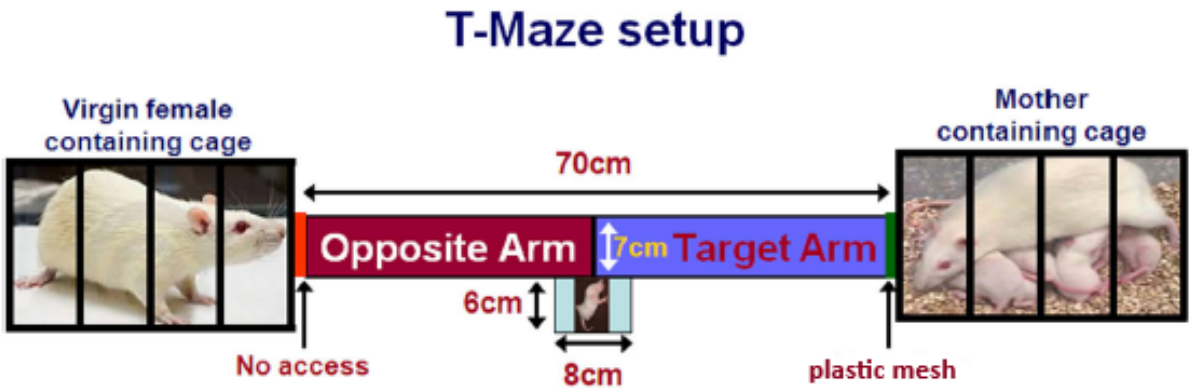

Figure 6. Schematic representation of the novel early experience model involving neonatal learning of a T-maze during PND 10-13, using denial of maternal contact as an event with negative emotional valence [14].

2.2.2. Food anticipation test

To measure the establishment of anticipatory behavior, we designed a two-phase food anticipation task (FA) [141], where rats had time-restricted access to a limited quantity of standard food chow (two-third of *ad libitum* daily feeding quantity, ~40g per three rats). For this test, we used an open field arena consisting of a square wooden box with a black painted surface (57 × 57 × 57cm), with an open top; an empty cylindrical food container (8.5cm diameter × 0.6cm height) was placed at a certain corner. On the first day of the FA test (PND 87-Accomodation day), each rat was color marked on its back to facilitate digital tracking, and all food from the home cage was removed. No additional food was added inside the home cage for the rest of the FA days. Before every FA test, all cage mates were weighted, and color marked and then simultaneously placed inside the arena. The rats were free to explore the arena for 30min (context-dependent learning period, CoP). At the end of the CoP period, the food container was removed for 2min; removal of the food container signified the

initiation of the cue-dependent learning period (CuP). Immediately after the end of the CuP period, the food container filled with 40g of food was returned to its original position in the arena. The animals were left undisturbed to feed for 30min inside the arena. At the end of the trial, the rats were returned to their home cage. The arena along with the food container was thoroughly cleaned with 70% ethanol. Leftover food in the arena was retrieved and measured. Water remained available *ad libitum* in the home cages. The food anticipation task took place for 8 consecutive days for each animal group. The first day was designated as day 0 and served as an accommodation trial. Daily weight loss was calculated as the ratio between daily weight and starting weight on day 0 (PND 87) [142]. On the final day (day 7, PND 94), the animals were sacrificed immediately after the end of the CuP. The animals were deeply anesthetized using isoflurane vapors, decapitated, and their brains were removed. After removal, the brains were snap-frozen in −35^{o}C isopentane and stored at −80^{o}C. Brains from basal animals of both groups (CTR and DER) were isolated at similar ages.

Every trial was recorded using a digital video camera with auto exposure and autofocus features disabled and zoom manually fixed. EthoVision 7XT (Noldus, Netherlands) software was used to digitally assess recordings of the food anticipation trials. The arena was split into four equal quadrants, and the quadrant containing the food container was designated as the feeder quadrant. For multiple animal tracking in food anticipation trial footage, multi-animal tracking setting was applied, and the color marker center point was chosen for animal identification. To smoothen small movement fluctuations, we filtered out movements that were less than 0.5cm (nesting-minimum distance moved). The software measured the total distance moved and the time spent inside the feeder quadrant. Rearing activity was manually assessed from each recording by three independent observers, and the average was calculated; the feeder quadrant rearing rate was calculated by dividing rearings inside the feeder quadrant by the total rearings measured.

2.2.2.1. Food access priority scoring

This behavioral parameter has been determined by Ermis Ryakiotakis and is described herein as it has been used in the statistical analysis. No further data will be presented in the current text.

To assess the formation and maintenance of a hierarchical food access order, during food restriction, we devised a scoring system (food priority score) that classified each rat according to the order of initial access to the feeder on each day of food anticipation testing. The first rat that reached the feeder was attributed 1 point, the second 2 points, and the third 3 points. The animals were scored

accordingly for each training day. Score values for the 7 days ranged between 7 and 21. By dividing the difference between the maximum and minimum score by 3, we calculated three scoring ranges: a dominant range of 7 to 11.66, an intermediate range from 11.67 to 16.33, and a subordinate range of 16.34 to 21. The animals of each group were distributed to a range according to their total score from all seven recorded trials (Figure 7).

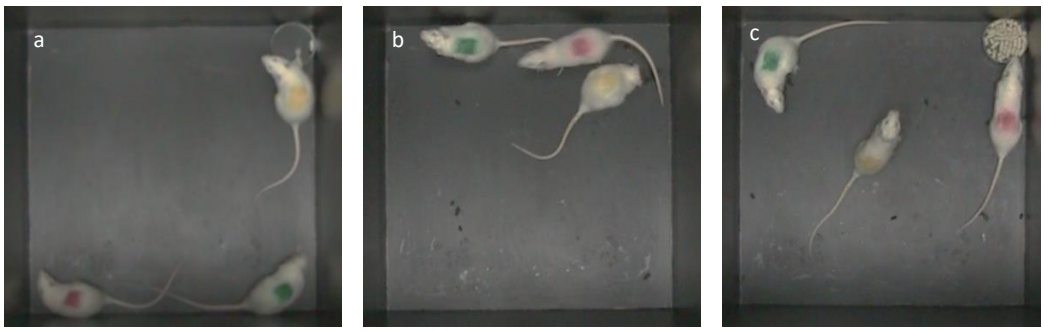

Figure 7. Food anticipation trial. (a) Context-Dependent trial: Empty food container present for 30 min. (b) Cue-dependent trial: food container was removed for 2min (cue), then food container filled with standard food was returned (c) Feeding and food access order evaluation for 30min.

2.3. Biochemical experiments

2.3.1. Western blotting

From the left hemisphere, ventral PFC (anterior–posterior: 3–6.12mm, rostral to bregma and from dorsal–ventral: 6mm and below, following olfactory bulb and projection removal) and nAC (anterior posterior: 1–3mm) [148] of each animal were homogenized in lysis buffer (100 mM, Tris-HCl pH:7.5, 250mM NaCl, 48mM, NaF 2mM, Na_3V0_4, 0.5% Triton X-100, 1:250 protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), 2mM dithiothreitol, 50mM ethylenediaminetetraacetic Acid), sonicated, and centrifuged at 15000rpm for 30min. The supernatant was collected, and the total protein quantity was determined by 280nm absorption measured on NanoDrop One (Thermo Fisher). Each Western blot sample was prepared by mixing 65µg of total protein with NuPAGE lithium dodecyl sulfate (LDS) sample buffer (Thermo Fisher) and 100mM dithiothreitol (Merck). Samples were heated at 75°C for 20min and then were loaded and separated on 4–12% gradient NuPAGE Bis-Tris precast polyacrylamide gels (Thermo Fisher) at 0.06A constant current using NuPAGE MOPS running buffer (Thermo Fisher) for 1h and 30min. Proteins were then transferred from the gel to a nitrocellulose membrane (0.45µm nitrocellulose paper Macherey-Nagel, Germany)

by applying a constant voltage of 30V for 2h, using the Surelock-X NuPAGE device and NuPAGE transfer buffer (Thermo Fisher). After transfer, the membranes were incubated in ponceau solution (0.1mM ponceau (Serva), 0.05% CH_3COOH) for 1min to assess transfer efficiency. Following ponceau staining, the membranes were washed with ddH_2O until stained lanes faded. Membrane lanes at 35–40 and 80–115 kDa were blocked in 10% normal goat serum or 10% normal rabbit serum TBS-T (0.05% Triton-X 100) respectively. Then, membrane lanes at 35–40 kDa were incubated with anti-GAPDH mouse monoclonal antibody, 1:1000 (Millipore) overnight at 4°C, while membrane lanes at 80–115 kDa were incubated with anti-dopamine receptor 1 (D1R) goat polyclonal antibody, 1:500 (Santa Cruz Biotechnology), for 48h at 4°C. The following day, membranes lanes at 35–40 kDa were incubated with goat anti-mouse polyclonal HRP-conjugated antibodies, 1:100000 (Millipore) for 2h at room temperature. Two days after the incubation with anti-D1R primary antibody, membrane lanes at 80–115 kDa were incubated with rabbit anti-goat polyclonal HRP-conjugated antibodies, 1:100000 (Millipore) for 2h at room temperature. Luminescence was induced using Immobilon Crescendo (Millipore) substrate. The membranes were exposed to autoradiographic films (Kodak XAR). After exposure, films were developed using Dentus D developer (Agfa, Belgium), fixed using Dentus F (Agfa), washed with dH_2O, dried, and scanned with a CanoScan8000F scanner. The images were produced by the PhotoStudio 5 (ArcSoft) software using the grayscale method with 1200dpi resolution and saved using the TIF format. Protein band optical density was determined using the ImageJ v.1.53 (NIH) software. D1R protein levels were assessed relative to GAPDH levels by calculating their ratio (D1R/GAPDH). The mean of D1R/GAPDH ratios from CTR samples was used as the reference baseline for each sample of every gel. To compare the groups between conditions, gels containing CTR samples from each condition were used. The mean basal CTR D1R/GAPDH ratio was used as the baseline and the experimental CTR/basal CTR ratio served as a normalization factor for every experimental measurement.

2.3.2. Autoradiographic *in vitro* binding

Autoradiographic *in vitro* binding was performed as described in Raftogianni et al., 2014 [101], (Figure 9). Briefly:

2.3.2.1. Tissue preparation

For autoradiographic *in vitro* binding adult male rats (PND89) of basal condition of both experimental groups (DER and CTR) were used. Animals were deeply anesthetized using saturated isoflurane gas, decapitated and their brains were isolated and frozen in -40°C isopentane. Brain tissues were cut into coronal 15µm sections on a cryostat (Leica CM1900, Nussloch, Germany) at 17°C, collected on silane-coated slides and stored at -80°C until further processed.

2.3.2.2. Receptor autoradiography – localization and quantification of dopamine receptor 1 (D1R)

For the determination of D1R binding density, autoradiographic *in vitro* receptor binding was performed with the D1R-specific radioligand, the ^{3}H-SCH23390 (NET-930 SCH-23390), (*N*-Methyl-^{3}H), with specific activity 85.0 Ci/mmol (PerkinElmer, Boston, MA02118, USA). More specifically, sections were pre-incubated in Tris–HCl buffer (50mM Tris–HCl, 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ and 1mM $MgCl_2$, pH 7.4) for 1h at room temperature (RT). Then, sections were incubated for 1h at RT in the above-mentioned assay buffer containing 5nM ^{3}H-SCH23390 and 100µM ketanserin. Nonspecific binding was evaluated in the presence of 1µM flupenthixol. Following incubation, slides were washed 2 x 5min in ice-cold assay buffer, dipped in ice-cold dH_2O and then dried under a stream of cold air.

For the autoradiography of D1R, tritium-sensitive films (MS-I, Kodak, Rochester, NY 14650, USA) were exposed to labeled dried sections, along with plastic [^{3}H]standards (American Radiolabeled Chemicals Inc., St. Louis, MO 63146, USA) for 6 weeks at 4°C. All films were manually developed for 3min in Kodak D-19 Developer (Kodak, France), fixed for 10min in sodium fixer (Kodak, France), washed under running water, rinsed in dH_2O and air-dried.

2.3.2.3. Quantitative analysis of autoradiographic images

Autoradiographs were scanned using a Cannon scanner (CanoScan 8000F, Canon, Japan) and quantitative analysis was performed using Scion Image 1.9.1 (Scion Corporation, Frederick, MD 21701, USA). Binding levels were quantified as optical density (OD) in selected brain areas: vPFC and nAC, as these regions constitute parts of the mesofrontal/mesolimbic system. The mean optical density was converted to receptor density (fmoles/mg tissue) based on the co-exposed ^{3}H-labeled standards. For each brain area, three to four sections, depending on the brain area, from each animal, spaced by 90µm along the rostrocaudal axis, were quantified. Specific binding was > 95% of the total binding. To better visualize and discriminate between structures and boundaries, adjacent

brain sections were stained with Cresyl Violet. Brain areas were identified using the adult rat brain atlas by Paxinos and Watson [143], (Figure 8).

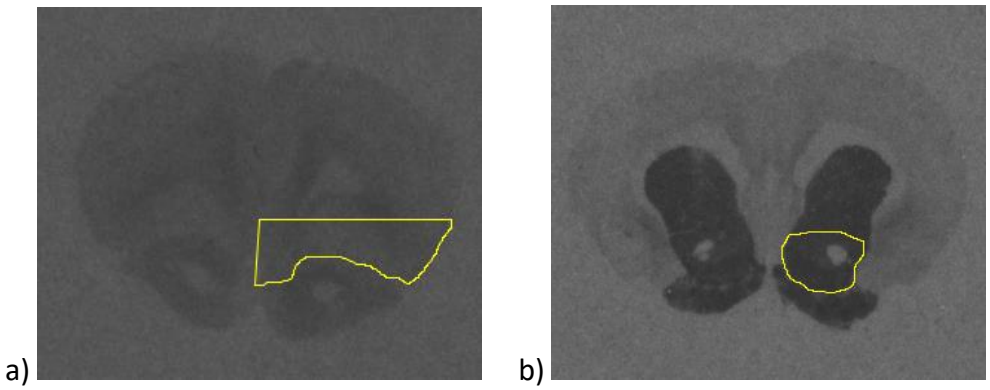

Figure 8. Autoradiography in vitro binding: Drawing D1R binding density in Image J. Lines indicate (a) the prefrontal cortex and (b) nucleus accumbens regions where quantification of D1R has been performed.

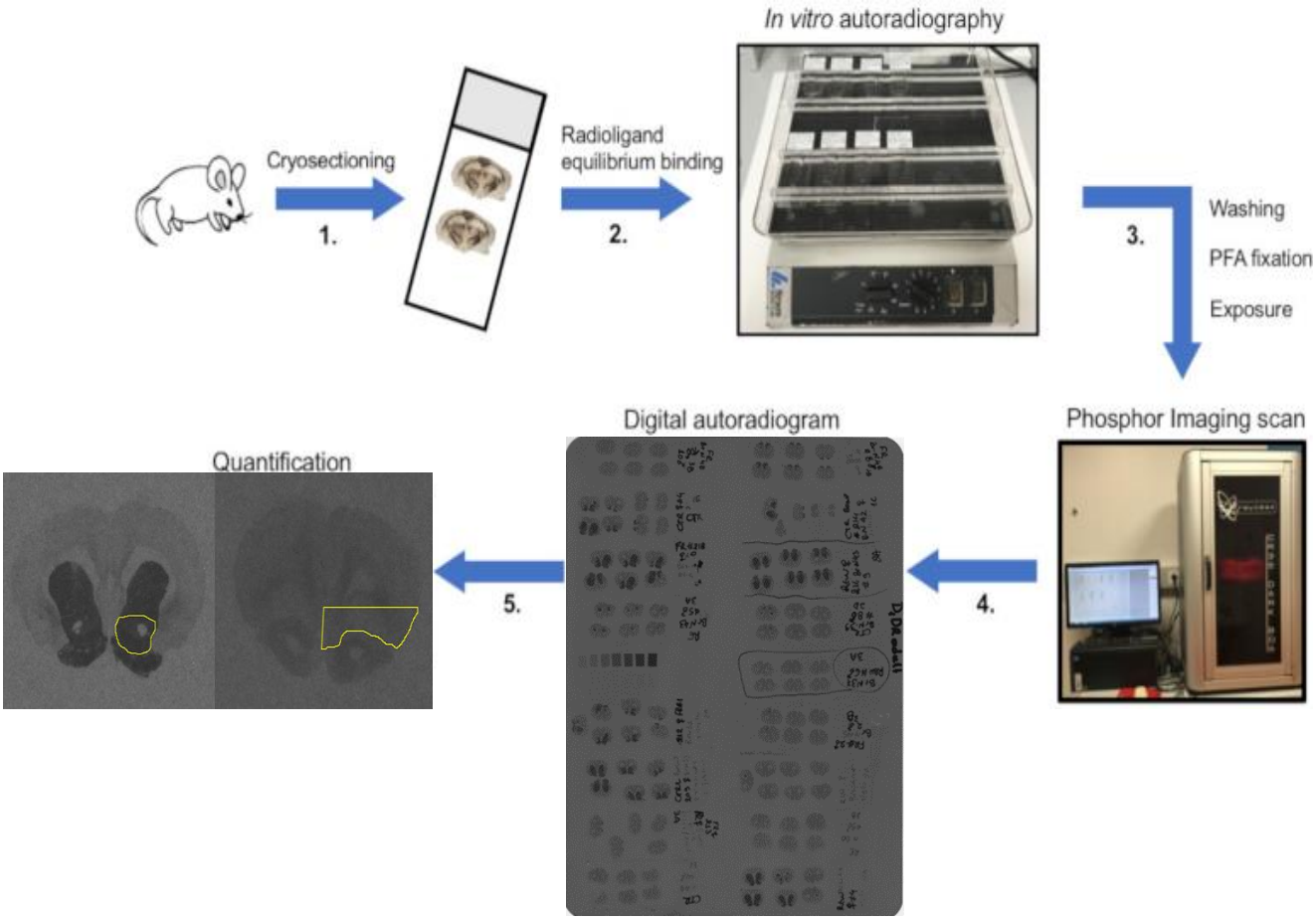

Figure 9. Schematic overview of the protocol of *in vitro* autoradiography binding [144].

2.3.2.4. Photomicrograph production

For Western blots and D1R autoradiograms, composite photomicrographs were produced with the Adobe Photoshop CS2 (Adobe Systems, San Jose, CA 95110, USA).

2.4. Statistical analyses

For statistical comparisons, we used the SPSS software (IBM) v.26. For our comparisons, we grouped our data sets by groups (CTR vs. DER), litter, condition (basal vs. experimental), and timepoint (days) where applicable. Behavioral parameters measured over time were analyzed by generalized estimated equations (GEEs). Each time-point (day) was set as a within-subject factor (repeated measures). In this analysis, we modeled using group as an independent factor, group (litter) as a nested factor, day as a repeated measure, and the interaction of group × day, followed by post hoc Bonferroni sequential test when appropriate. In every GEE comparison, the food priority score and weight loss were factored in as covariates. According to the literature, we considered that food access priority influenced the behavioral output of the next day [145]. Consequently, the daily food priority score was factored in as a covariate in the analysis of the behavior of the next day. If group × day interactions were found, we performed post hoc GLM for each time-point separately with group and group (litter) modeled together with food priority score and weight loss factored in as covariates.

To identify the potential effects of the DER experience on D1R protein levels during the experimental manipulations, we used a generalized linear model (GLM), where we modeled using as independent factors group (CTR, DER) and condition (Basal, FA) as well as their interaction group × condition and as a nested factor group (litter). When group × condition interaction was found, post hoc Bonferroni sequential comparisons were made between groups for each condition (basal CTR vs. basal DER, and experimental CTR vs. experimental DER) and between conditions for each group (basal CTR vs. experimental CTR, and basal DER vs. experimental DER).

To identify the potential effects of the DER experience on D1R binding levels under basal conditions, we used a generalized linear model (GLM), where we modeled using as independent factor the group (CTR, DER) of animals and as a nested factor group (litter).

For all tests, differences were considered significant when a p-value = < 0.05.

3. Results

3.1. Behavioral Assay: Food anticipation task

The DER experience alters the preference for the area where the food reward is expected to be delivered.

The food anticipation (FA) trials explore the effects of the DER experience on the anticipatory behavior of the animals when a reward in the form of food is expected to be delivered. These trials are designed to record the learning progression and efficiency of two distinct learning modules: contextual and cue learning. Thus, each trial initiates with a 30-min context-dependent learning period followed by a 2-min cue-dependent learning period where the removal of the empty food container acts as a reward-predicting cue.

3.1.1. Context-dependent learning trial

Studying mobility during the context-dependent learning period (CoP) can reveal critical components of anticipatory behaviors such as hypermobility and increased exploration of the area where food is expected to be delivered. The changes in the distance moved and rearing activity across the days of FA training can be correlated with anticipatory responses. General locomotion increased steadily across training days, verifying an anticipatory effect. GEE covariate analysis showed a significant daily weight loss effect on total distance moved ($W = 6.76$, $p = 0.009$). Additionally, GEE analysis of the total distance moved and rearing activity during the CoP revealed significant group × day interactions ($W = 21.75$, $p = 0.001$ and $W = 46.85$, $p < 0.001$), indicating horizontal and vertical locomotion differences between the groups on certain training days. Post hoc analysis of the total distance moved showed that the DER group displayed lower locomotion on day 2 ($W = 5.29$, $p = 0.021$) compared to the CTR group (Figure 10). Post hoc analysis of the total rearings indicated that the DER group displayed higher rearing activity on days 3 ($W = 14.95$, $p < 0.001$) and 6 ($W = 6.1$, $p = 0.013$), while lower rearing activity on day 7 ($W = 7.82$, $p = 0.005$) compared to CTR (Figure 11).

Increased time spent and rearing rate inside the quadrant where the empty food container is located can act as an indicator of context-reward association. GEE covariate analysis showed a significant food priority score effect both on relative time spent ($W = 6.96$, $p = 0.008$) and rearing rate ($W = 5.66$, $p = 0.02$) inside the feeder quadrant. Moreover, GEE analysis showed a group × day interaction on the relative time spent ($W = 25.93$, $p < 0.001$) and rearing rate ($W = 13.19$, $p = 0.04$) in the quadrant where food was later delivered, indicating a difference between the groups in the development of preference and targeted exploration for the quadrant where food delivery was expected: DER rats' preference for and rearing rate in the rewarded quadrant appeared lower than normal. Post hoc analysis of the relative time spent in the feeder quadrant, for each day, indicated that DER animals spent less time in the quadrant where food was delivered on days 3 ($W = 17.86$, $p < 0.001$), 4 ($W = 9.40$, $p = 0.002$), 5 ($W = 4.67$, $p = 0.031$), and 7 ($W = 11.349$, $p < 0.001$) (Figure 12), suggesting an impaired context-reward association by the DER rats. Similarly, post hoc analysis of the rearing rate in the feeder quadrant showed lower rate by the DER animals on days 3 ($W = 15.16$, $p < 0.001$), 4 ($W = 6.13$, $p = 0.013$), 6 ($W = 4.0\ 5$, $p = 0.044$), and 7 ($W = 7.7$, $p = 0.006$) (Figure 13). Interestingly, while DER animals did not exhibit major alterations on general anticipatory mobility and exploratory activity, they displayed lower preference and exploration rate of the quadrant containing the empty food container.

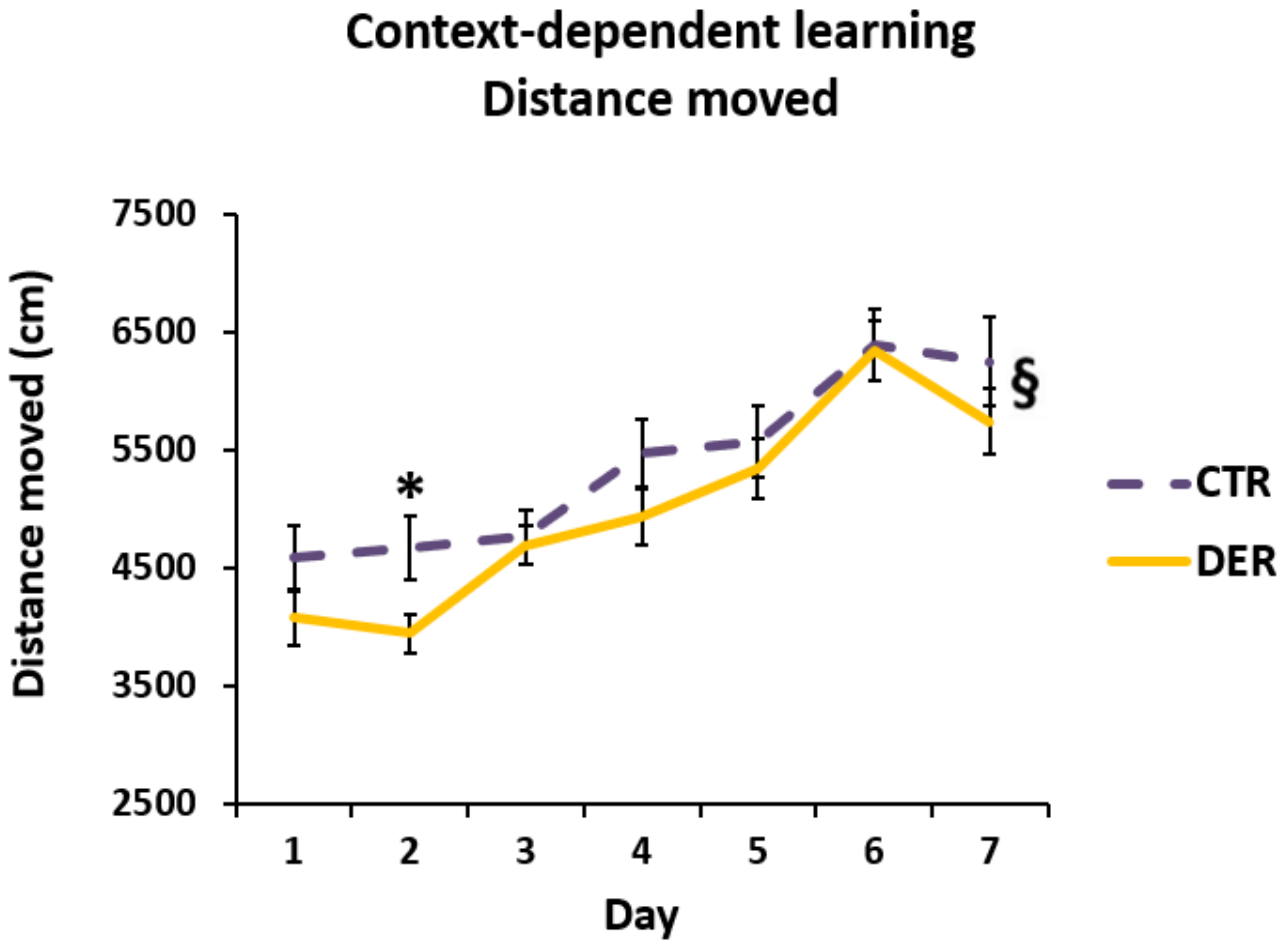

Figure 10. Behavior during the context-dependent learning period of FA training: Mobility differences between the groups during training days. Total distance moved (cm) in the open field arena during the 30-min context-dependent learning period for each day (one trial/day). On day 2 of FA training, DER animals showed reduced mobility. Lines represent means ± SEM. (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

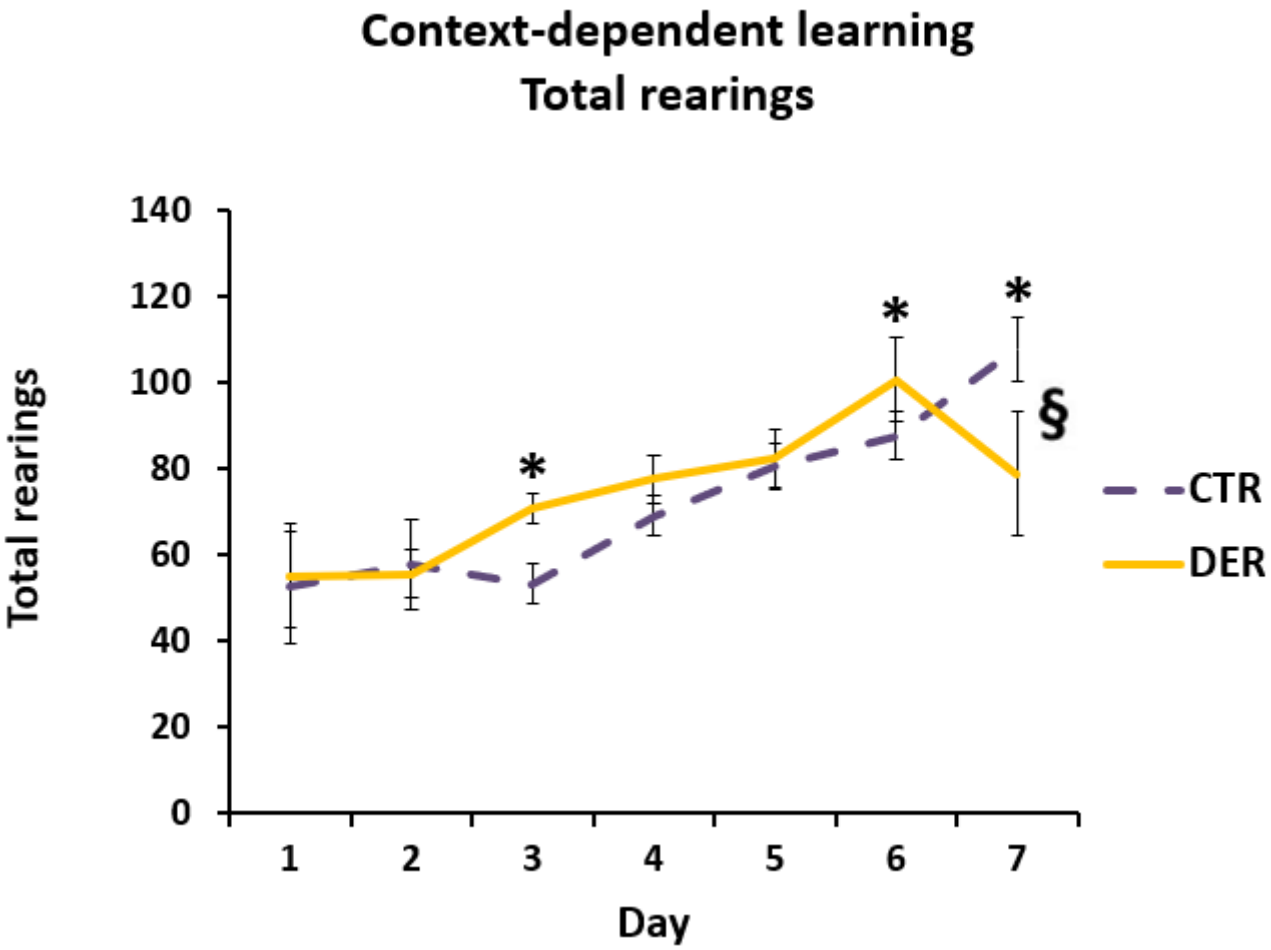

Figure 11. Behavior during the context-dependent learning period of FA training: Rearing activity differences between the groups during training days. Total rearings performed during the 30-min context-dependent learning period for each day (one trial/day). On days 3 and 6, DER animals displayed increased rearing activity, while on day 7 this was lower, compared to CTR. Lines represent means ± SEM. (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

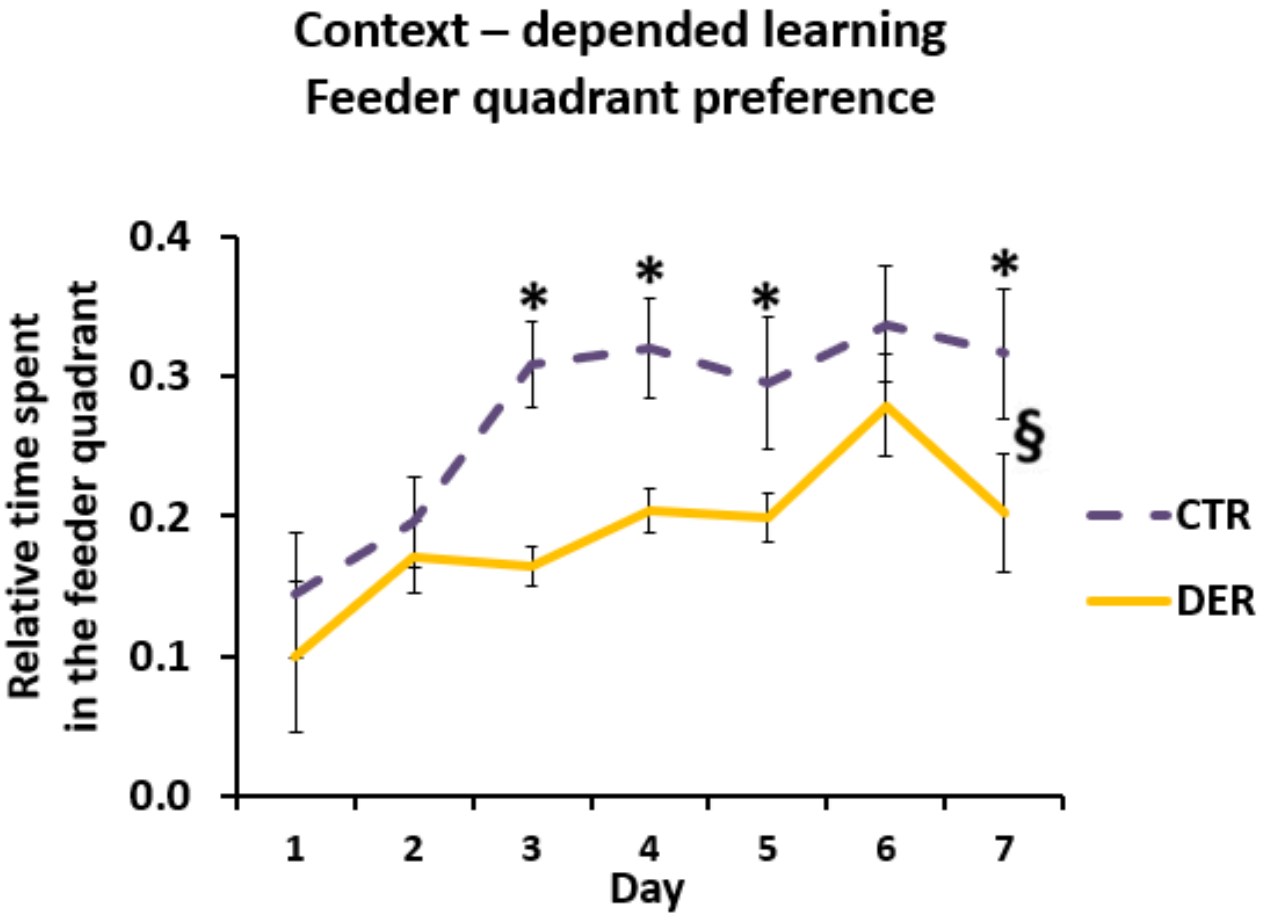

Figure 12. Behavior during the context-dependent learning period of FA training: Relative time spent in the rewarded quadrant of the open field (quadrant containing the empty food container in which food will be provided after the end of the anticipation training), during the 30-min context-depended learning period for each day (one trial/day). Relative time was calculated by dividing the time spent inside the rewarded quadrant by the total time of the context-dependent learning period. DER animals preferred less the rewarded quadrant on days 3, 4, 5, and 7 of FA training compared to CTR. Lines represent means ± SEM (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

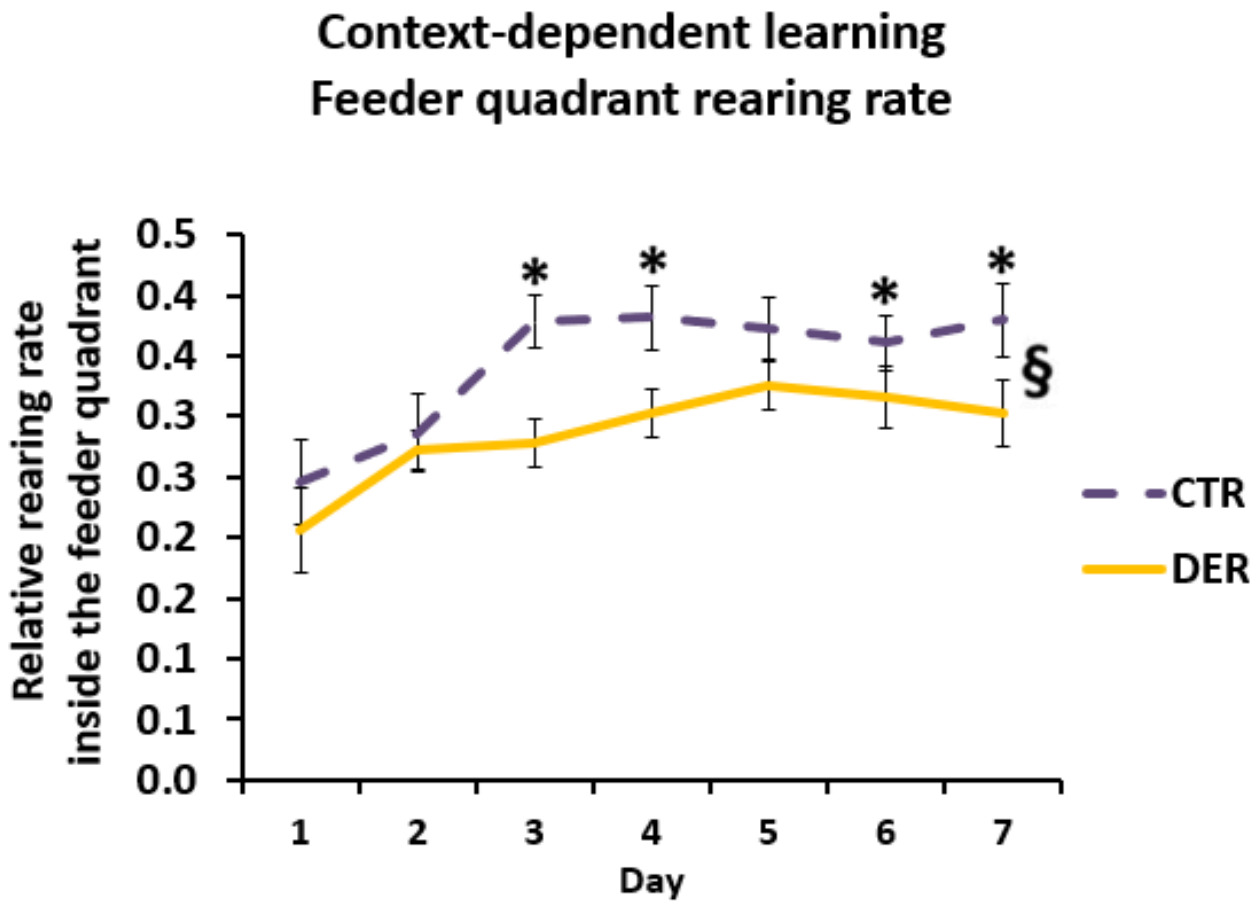

Figure 13. Behavior during the context-dependent learning period of FA training: Rearing rate inside the rewarded quadrant of the open field (quadrant containing the empty food container in which food will be provided after the end of the anticipation training), during the 30-min context-depended learning period for each day (one trial/day). Rearing rate was calculated by dividing rearings inside the feeder quadrant by the total rearings measured. DER animals displayed a reduced rearing rate inside the rewarded quadrant on days 3, 4, 6, and 7 of FA training compared to CTR. Lines represent means ± SEM (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

3.1.2. Cue-dependent learning trial

Studying the behavioral responses following the removal of the food container (CuP) allowed us to investigate the DER effects on cue-reward conditioning (Pavlovian dependent–independent cue association). GEE covariate analysis indicated a significant daily weight loss effect both on distance moved ($W = 4.16$, $p = 0.041$) and total rearings during CuP ($W = 7.42$, $p = 0.006$), suggesting that weight loss influenced general anticipatory mobility and exploratory activity. In addition, GEE analysis revealed a group × day interaction both on the distance moved ($W = 34.59$, $p < 0.001$) and total rearings ($W = 36.82$, $p < 0.001$), suggesting differential mobility and exploratory activity

between the groups following the cue onset. Post hoc analysis showed that DER animals displayed increased distance moved on days 1 ($W = 10.66$, $p = 0.001$), 3 ($W = 10.33$, $p = 0.001$), and 4 ($W = 7.08$, $p = 0.008$) (Figure 14). Furthermore, post hoc analysis showed that the DER group displayed increased rearing activity on days 1 ($W = 7.32$, $p = 0.007$), 3 ($W = 30.827$, $p < 0.001$), 4 ($W = 37.62$, $p < 0.001$), 6 ($W = 4.66$, $p = 0.031$), and 7 ($W = 6.66$, $p = 0.01$) (Figure 15). DER rats appeared to respond with increased horizontal mobility after the removal of the feeder (cue) on the first days of FA, while after day 5, horizontal mobility levels were similar in both groups. Interestingly, DER animals displayed increased rearing activity on almost every FA test day suggesting a reinforced general exploratory response to the reward-predicting cue.

Regarding the relative time spent and the rearing rate inside the rewarded quadrant, GEE covariate analysis showed a significant food priority score effect on both parameters ($W = 9.68$, $p = 0.002$ and $W = 10.04$, $p = 0.001$). Furthermore, GEE analysis of the relative time spent and rearing rate inside the feeder quadrant revealed a group × day interaction ($W = 17.13$, $p = 0.009$ and $W = 22.29$, $p < 0.001$, respectively), suggesting a difference in the association rate of the quadrant where food was provided after the end of the anticipation period with the imminent food delivery. Post hoc analysis revealed that the DER rats spent less time within the rewarded quadrant on day 5 ($W = 5.46$, $p = 0.019$) (Figure 16) and showed a lower rearing rate inside this quadrant on day 7 ($W = 6.42$, $p = 0.011$) compared to the CTR (Figure 17). While in both groups preference for the rewarded quadrant gradually increased, on day 5, DER animals exhibited a delay in their preference increase. Nevertheless, from day 6, DER animals achieved a similar preference with the CTR. Notably, on day 7, the DER rearing rate inside the rewarded quadrant was lower than that of the CTR ones.

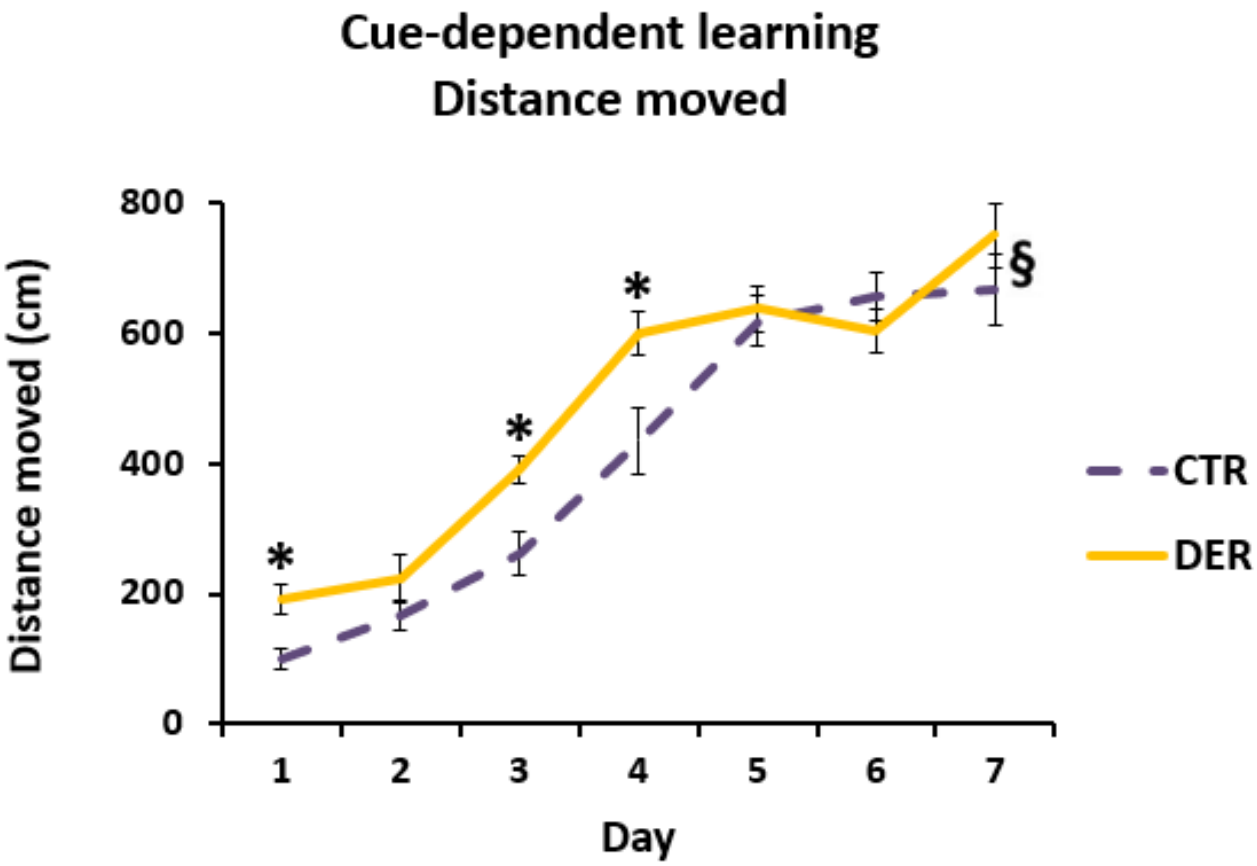

Figure 14. Behavior during the cue-dependent learning period of FA training: Mobility differences between the groups following the cue onset during training days. Total distance moved (cm) during the 2-min cue-dependent learning period for each day (one trial/day). DER animals showed increased mobility on days 1, 3, and 4. Lines represent means ± SEM. (CTR: n = 18, DER: n = 18). §: GEE Group X Day interaction, $p < 0.05$; *: post-hoc group effect, $p < 0.05$.

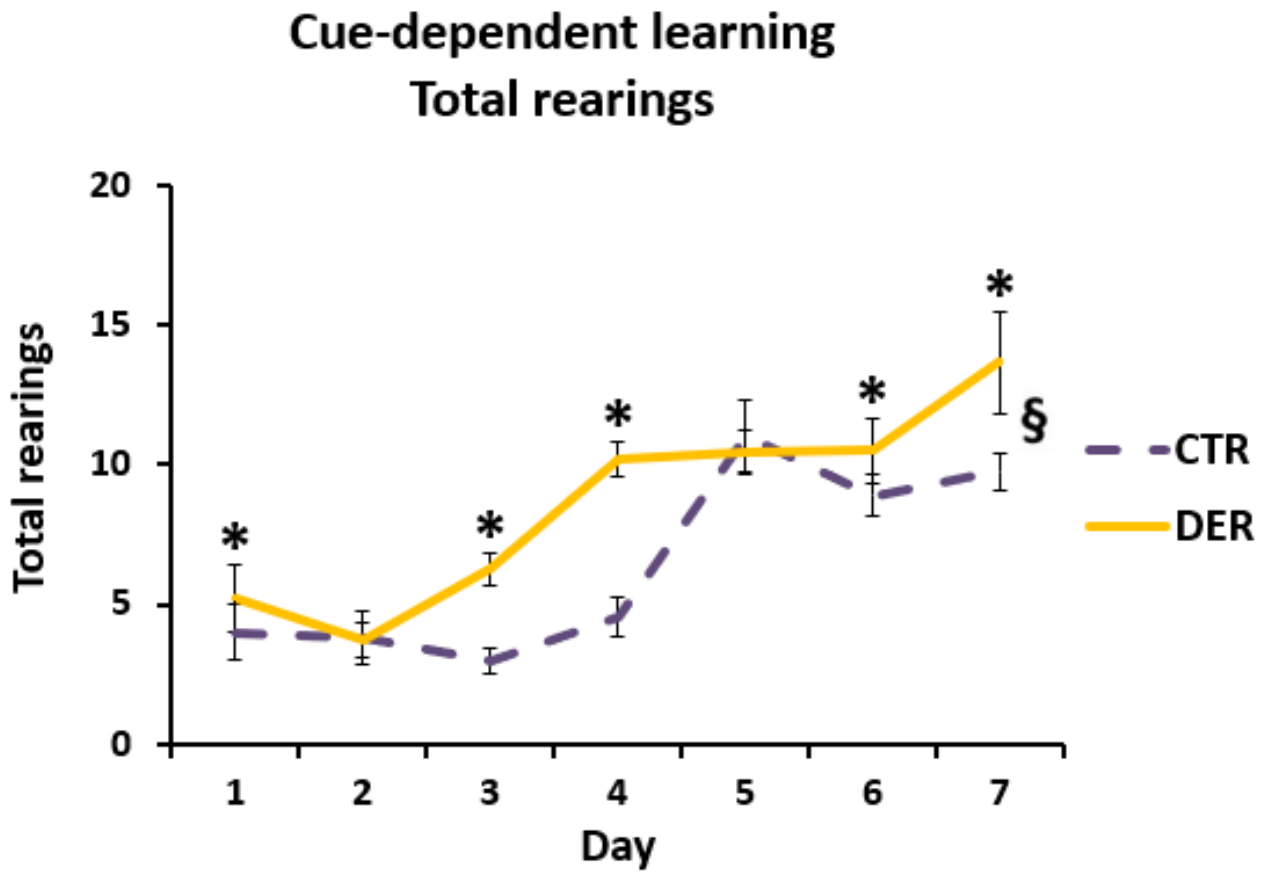

Figure 15. Behavior during the cue-dependent learning period of FA training: Rearing activity differences between the groups following the cue onset during training days. Total rearings performed during the 2-min cue-dependent learning period for each day (one trial/day). DER animals displayed increased rearing activity on days 1, 3, 4, 6, and 7 compared to CTR. Lines represent means ± SEM. (CTR: n = 18, DER: n = 18). §: GEE Group X Day interaction, $p < 0.05$; *: post-hoc group effect, $p < 0.05$.

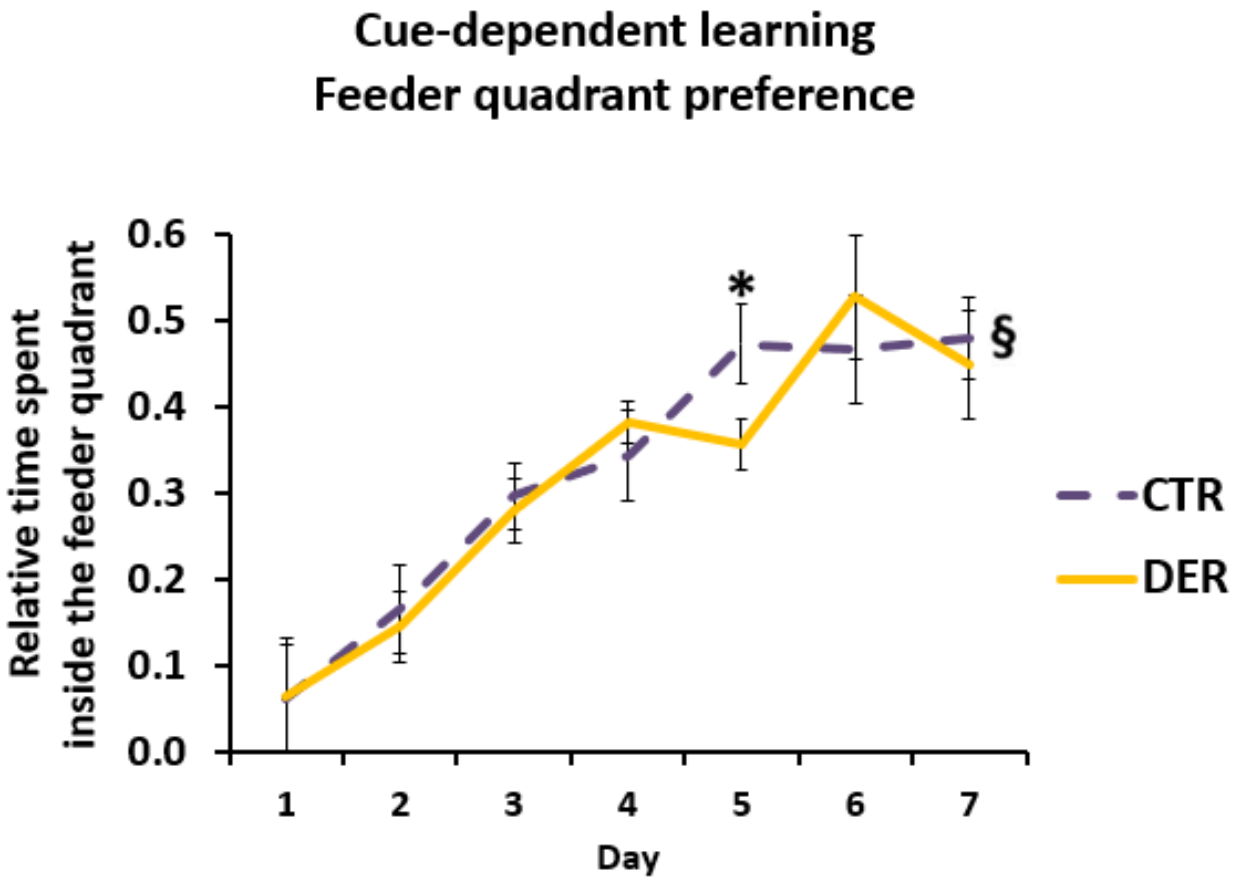

Figure 16. Behavior during the cue-dependent learning period of FA training: Relative time spent on the rewarded quadrant of the open field (quadrant where food will be provided after the end of the anticipation training), during the 2-min cue dependent learning period for each day (one trial/day). Relative time was calculated by dividing the time spent inside the rewarded quadrant by the total time of the cue-dependent learning period. DER rats preferred less the rewarded quadrant on day 5. Lines represent means ± SEM. (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

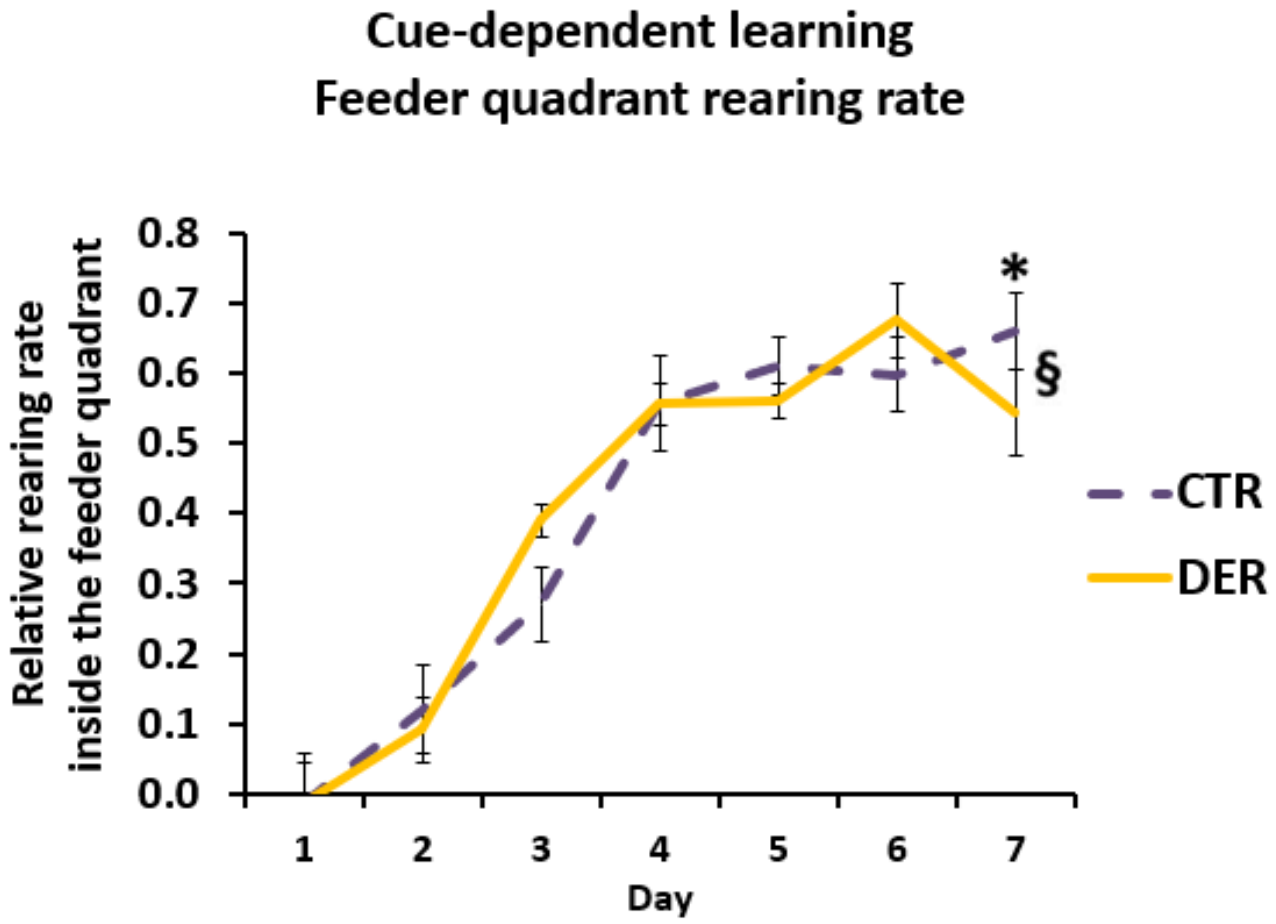

Figure 17. Behavior during the cue-dependent learning period of FA training: Rearing rate inside the rewarded quadrant of the open field in which food delivery is expected, during the 2-min context-depended learning period for each day (one trial/day). Rearing rate was calculated by dividing rearing inside the feeder quadrant by the total rearings measured. DER animals displayed a reduced rearing rate inside the rewarded quadrant on day 7 compared to CTR. Lines represent means ± SEM (CTR: n = 18, DER: n = 18). §: GEE group × day interaction, $p < 0.05$; *: post hoc group effect, $p < 0.05$.

3.2. Biochemical Assay: Dopamine Receptor 1 levels in vPFC and nAC

3.2.1. Western Blot analysis

DER experience affects dopamine receptor D1R protein levels in vPFC during basal conditions.

Food anticipation (FA) results indicated that the DER experience induces reward association deficiencies. To identify potential DER effects on the dopaminergic aspect of reward circuit activation during the FA trials, we measured dopamine receptor 1 (D1R) levels in the ventral prefrontal cortex (vPFC), and in the nucleus accumbens (nAC). GLM analysis showed a group × condition interaction in vPFC ($W = 5.010$, $p = 0.025$) (Figure 18). Basal DER animals showed increased D1R levels compared to both basal CTR and experimental DER ones (post hoc basal CTR vs. basal DER: $p < 0.001$, basal DER vs. experimental DER: $p = 0.02$), (Figure 18, 19). In nAC, there was also a group × condition interaction according to GLM analysis ($W = 4.792$, $p = 0.029$). Experimental DER animals showed increased D1R levels compared to both basal DER and experimental controls (post hoc basal DER vs. experimental DER: $p <0.001$, experimental CTR vs. DER: $p = 0.002$), (Figure 20, 21).

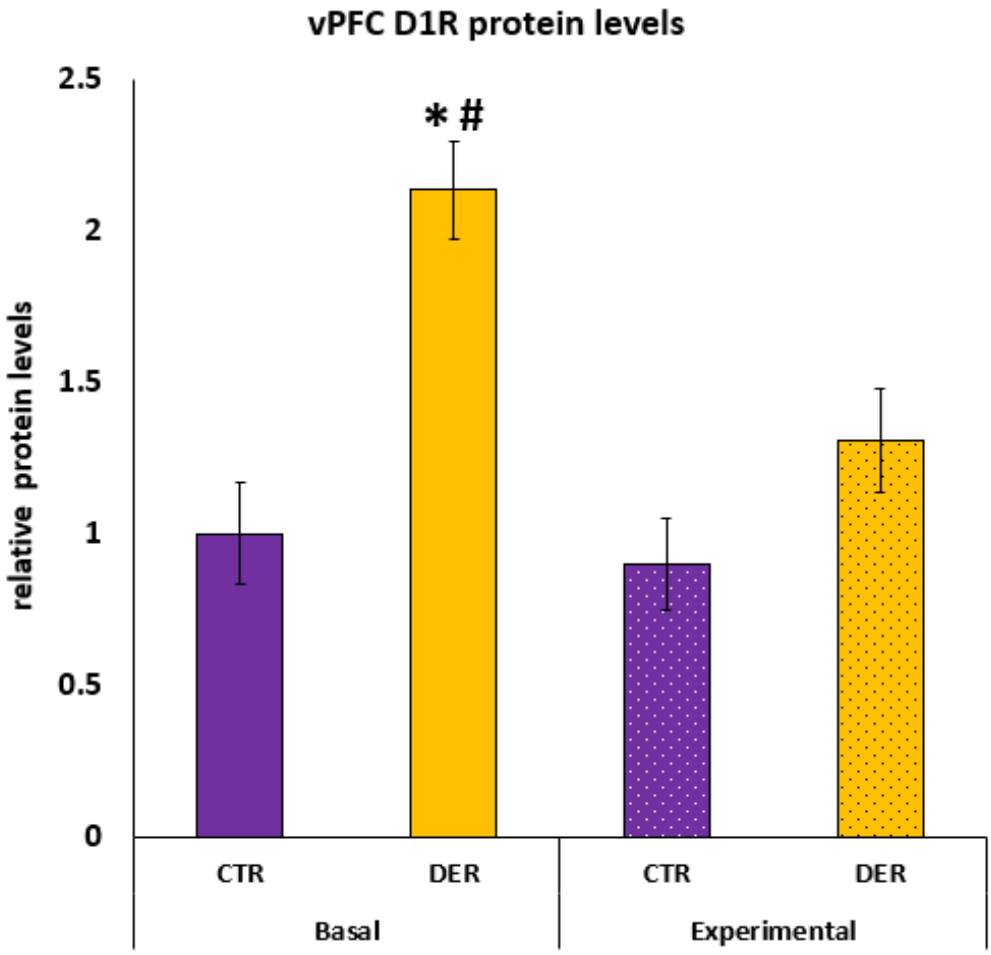

Figure 18. Relative protein levels D1R in vPFC determined by Western blotting. Protein levels were normalized using the basal CTR group as the baseline 100%. Adult DER animals showed increased D1R levels under basal conditions. Bar graphs represent means ± SEM. (basal CTR: n = 14, basal DER: n = 15, experimental CTR: n = 17, experimental DER: n = 14). *: post-hoc group effect (Basal CTR vs. Basal DER), $p < 0.05$, #: post-hoc condition effect (Basal DER vs. Experimental DER), $p < 0.05$.

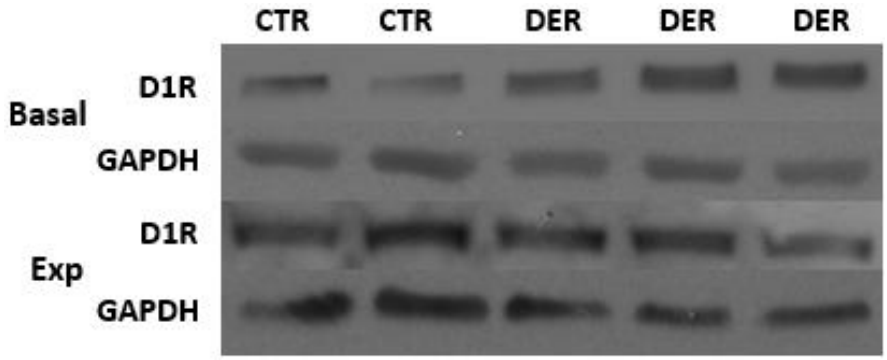

Figure 19. Relative protein levels determined by Western blotting: vPFC D1R protein levels were normalized using the basal CTR group as the baseline 100%.

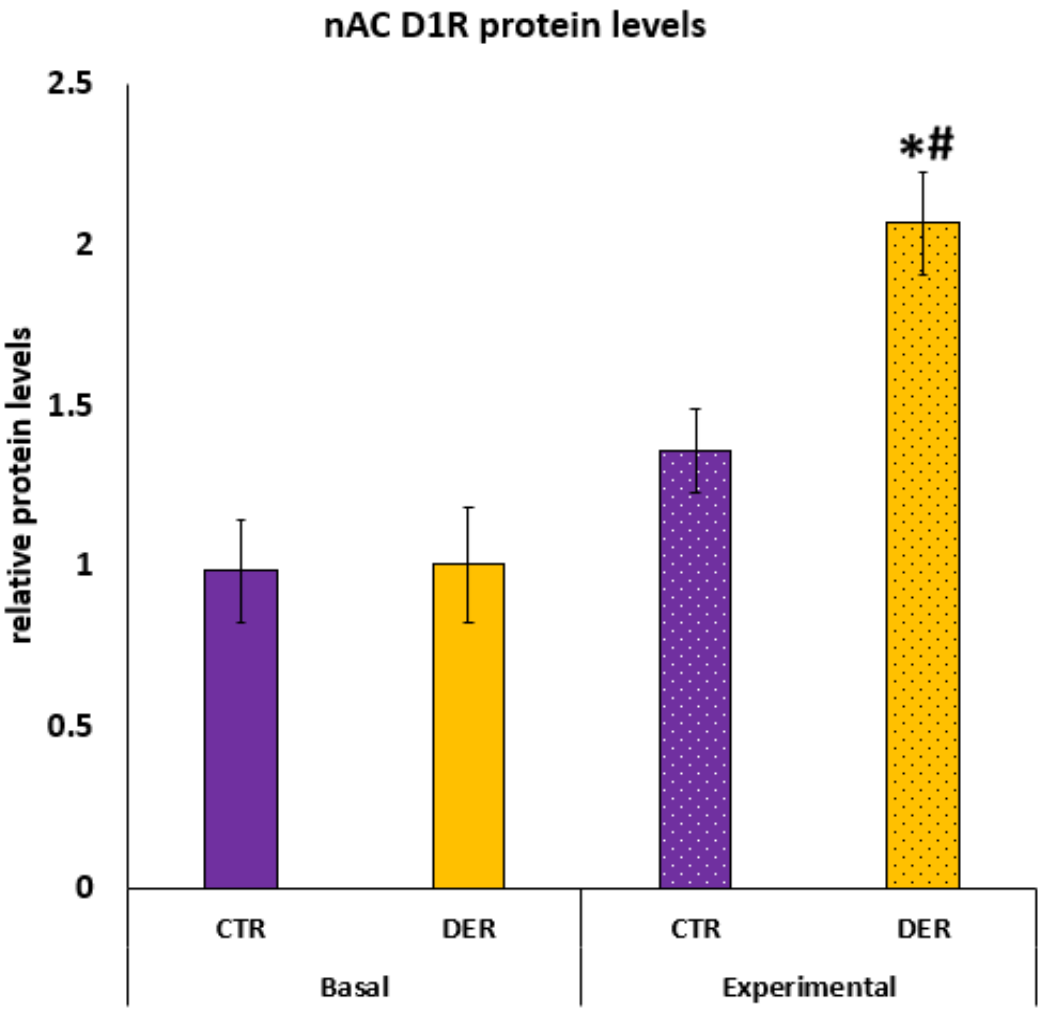

Figure 20. Relative protein levels D1R in nAC determined by Western blotting. Protein levels were normalized using the basal CTR group as the baseline 100%.Adult DER animals showed increased D1R levels under experimental conditions following 7 days of FA. Bar graphs represent means ± SEM. (basal CTR: n = 14, basal DER: n = 13, experimental CTR: n = 20, experimental DER: n = 18). *: post-hoc group effect (Experimental CTR vs. Experimental DER), $p < 0.05$, #: post-hoc condition effect (Basal DER vs. Experimental DER), $p < 0.05$.

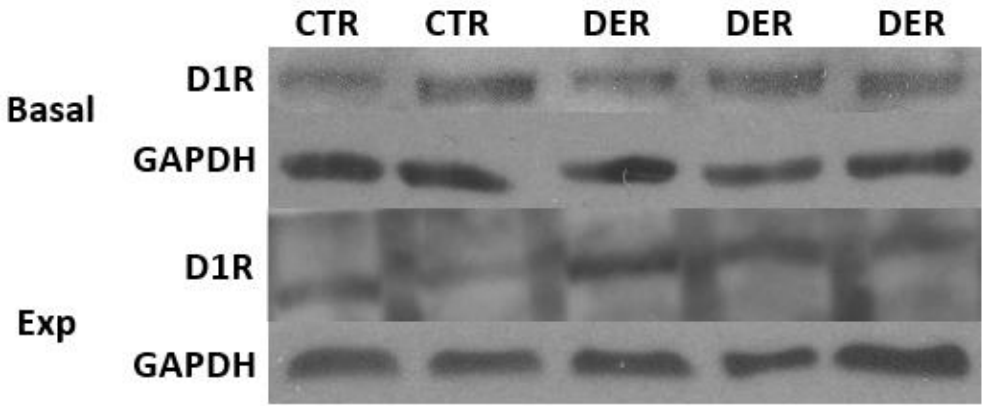

Figure 21. Relative protein levels determined by Western blotting: nAC D1R protein levels were normalized using the basal CTR group as the baseline 100%.

3.2.2. Autoradiography in vitro binding

DER experience decreases dopamine receptor D1R protein levels in nAC during basal conditions

To investigate the effects of DER experience on the dopaminergic aspect of reward circuit under basal conditions, we determined D1R receptor binding density performing autoradiographic *in vitro* receptor binding. GLM statistical analysis did not reveal statistically significant differences on PFC D1R between CTR and DER adult male animals (Figure 22).

In nAC, similar statistical analysis (GLM analysis) showed a significant group effect (W = 4.775, p = 0.029) on D1R protein levels under basal conditions, with adult DER animals showing lower D1R density when compared to the CTRs (Figure 23).

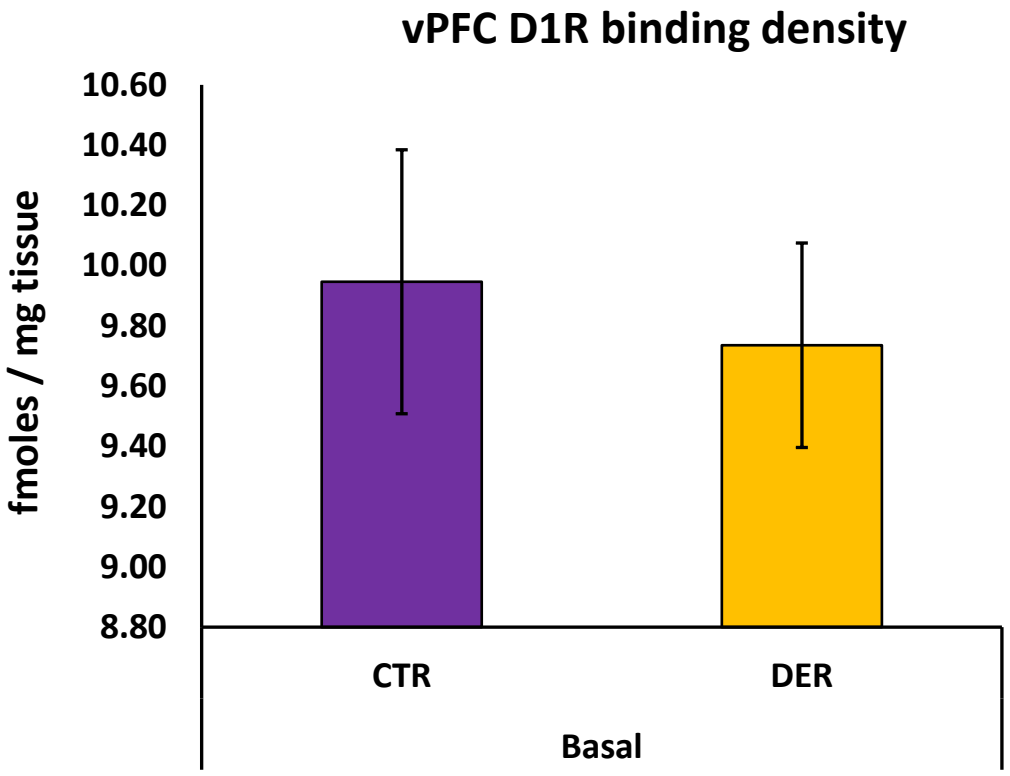

Figure 22. Effects of the DER early-life experience on basal levels of D1R receptor density in vPFC as assessed by autoradiographic *in vitro* binding. Bars represent means ± SEM of D1R receptor binding densities in fmoles per mg tissue (basal CTR: n = 3, basal DER: n =5).

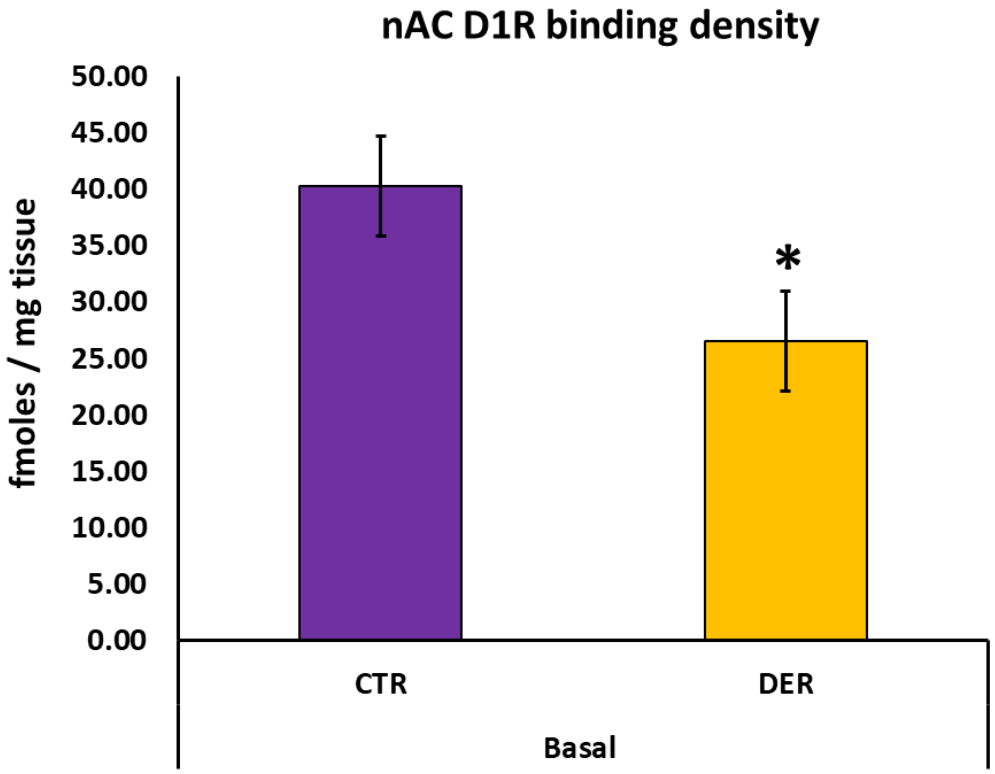

Figure 23. Effects of the DER early-life experience on basal levels of D1R receptor density in nAC as assessed by autoradiographic *in vitro* binding. Bars represent means ± SEM of D1R receptor binding densities in fmoles per mg tissue (basal CTR: n = 3, basal DER: n =3). Adult male DER animals showed decreased D1R levels under basal conditions. *: $p < 0.05$.

Methodological comment:

Data on D1R levels under basal conditions derived from Western blots and *in vitro* autoradiographic binding are not in line: For vPFC Western blots show increased levels of D1R in DER animals, a difference not detected by *in vitro* binding. Similarly, for nAC Western blots detect no difference while *in vitro* binding indicates lower levels in the DER group. This discrepancy could be explained based on the superior specificity of Western blotting compared to *in vitro* binding: The radioligand employed (^{3}H-SCH-23390) has a similar affinity for D1R and D5R receptors, while the antibody used in the Western blots is specific for D1R protein. We can postulate that *in vitro* binding detects two populations of dopamine receptors, a common one with Western blotting and an additional one, that of D5Rs. If this is verified (e.g. by Western blotting for D5R), our data could imply that under basal conditions, in both vPFC and nAC CTR animals have higher levels of D5Rs than the DER.

4. Discussion

In the present work we investigated the effects of a mildly adverse neonatal experience (DER training) on the emergence of anticipation for food-reward in adult male rats as well as on the dopaminergic system of vPFC and nAC, key areas of the brain reward network.

4.1. Investigating the effects of the DER experience in the development of anticipatory behavior in a context- and cue-dependent trial in the presence of cage-mates

The results revealed that there were significant differences between DER and CTR groups regarding the development of anticipatory behavior in a novel environment of an open field as well as food access priority following grouped FA training. During the context-dependent learning phase (CoP), food restriction and the resulting weight loss affected horizontal locomotion, while during the cue-dependent learning phase (CuP), they affected both horizontal and vertical activity (rearing behavior). In addition to these effects, the main discovery was that the DER animals had a deficiency in context-reward association. On the other hand, food access priority affected both the relative time spent and the rearing rate within the feeder quadrant during both phases (CoP and CuP). Apart from this, in the DER group there was also social instability in the food access priority after the food presentation in the open field.

The food anticipation (FA) training is an open field arena [146] which contains an unfamiliar object, specifically a feeder placed at a fixed corner of the arena. Results showed that during the context-dependent learning period (CoP) of the grouped FA training, DER animals displayed a mildly reduced locomotion activity along with exploratory behavior alterations. Unfamiliar novel objects are known to cause avoidance reactions in rats [147] and besides that early life stress (ELS) could increase the expression of such reactions [148]. Notably, it has been documented that mobility levels reduced in open field trials have been reported following social stress not only in control [149] but also in DER animals [150]. Previous work from our laboratory has shown that the DER experience induces passive behavioral responses under stressful conditions [151], hence it seems that the behavioral

responses of DER animals regarding decreased horizontal mobility on day 2 and decreased vertical mobility on day 7 of FA training were caused by increased stress. Novel and unfamiliar environment, food restriction and possibly social factors are considered to be factors causing stress during the FA grouped trials. Concerning food restriction, it leads to weight loss and increased motivation for food reward, while it can also induce stress responses [152, 153]. Regarding social factors, the presence of other animals in FA training, even cagemates, could also cause stress. On the one hand it is well established that social interactions reduce stress in general [139], but on the other hand they can also induce stress transmission [154, 155]. Additionally, findings from previous experiments in our laboratory [151] indicated that there were no alterations in locomotion activity of individually tested DER animals, not accompanied with other stressors. All these stressors, namely, novel object, food deprivation, and social stressor, could act synergistically on the animals resulting to the reduced mobility and feeder quadrant avoidance exhibited by the DER animals. These findings provide for the first time information in respect to the adverse effects of the DER experience during novelty/stress exposure in groups.

According to our findings, during the context-dependent learning period (CoP), it seems that the FA training could effectively induce anticipatory hyperactivity in DER and CTR animals, as horizontal locomotion activity from day 3 in FA trial did not differ between both groups, and increased progressively, as did the number of rearings. Indeed, we demonstrated that weight loss affected horizontal mobility levels, a finding which agrees with other studies that have linked food deprivation with high locomotion levels [156, 157]. At the same timeframe, both DER and CTR feeder preference levels increased but contrary to locomotion, DER animals generally spent less time and displayed lower rearing rates inside the feeder quadrant compared to CTR. An interesting observation is that the DER rearing rate inside the feeder quadrant was lower than CTR even on days when DER animals displayed increased total rearings. This indicates that DER experience is associated with reward association deficiencies and contextual memory impairments. Problems regarding contextual memory have already been described previously in other models of early life adversity [158, 159]. It has also been reported that stress can trigger depression-like mental disorders which possibly affect reward learning [160] and disrupts associative memory according to results from human [161] and animal studies [162, 163, 164]. The DER-driven reward associative memory deficiency may be caused by the stress of novelty stated earlier in combination with the stress of food restriction. Additionally, the statistical analysis highlighted food priority access as an important factor affecting the development of feeder quadrant preference. Thus**,** the increased

social instability (discussed below) may contribute to the etiology of the reward association deficits observed in DER animals during the CoP, as social conflict is a well-known stressor [165].

During the cue-dependent learning period (CuP), DER animals displayed increased locomotion compared to CTR on days 1, 3, and 4, as well as increased rearing activity on most days following feeder removal (cue). Furthermore, we found that both the total distance moved, and total rearings were also influenced by daily weight loss. However, DER preference for the quadrant where the food was expected to be delivered was lower on day 5, and the rearing rate inside the same quadrant was lower on day 7. Analogous to the CoP, both behaviors were influenced by food priority scores. Both horizontal and vertical locomotion levels increased progressively for both groups. Both groups developed behavioral reactions to the food-predicting cue that closely resemble Pavlovian conditioning [166], such as increased locomotion and reward place preference after cue presentation. The increased general mobility of the DER animals on day 1 could result from stress-relieving effects following feeder removal. On the other hand, the higher locomotion activity of DER animals on later days, as well as the increased rearing activity could reflect a reward oversensitization due to food restriction [167, 168]. Despite their general vertical and horizontal locomotor over-sensitization, DER rats displayed delayed preference acquisition and lower rearing rate targeted to the rewarded quadrant on day 7, compared to CTRs, indicating a reward place association deficiency. It should be noted that DER quadrant preference reached CTR levels after day 6, and the rearing rate fell below CTR levels on day 7, despite their higher total rearing count. As discussed above, DER experience is associated with spatial reward association deficiency. Although DER animals learned to expect the arrival of the cue-predicted reward, they had difficulty forming a spatial-reward association. Similar effects of short-term spatial memory disruption have already been described previously in other models of early life adversity [169]. These findings support the hypothesis that the spatial deficiency induced by the DER experience during both CoP and CuP of FA training could be stress related. Alternatively, it is possible that elevated stress of DER animals forces them to develop an alternative reward driven strategy that favors increased exploratory activity rather than a reward area-focused response. These observations have been found in mice where acute stress led them to a shift from a spatially focused strategy to a stimulus response one [170].

4.2. Exploring the impact of DER experience in the stability of food approach priority during reward delivery

FA training is a group task where three cage mates learn together to anticipate food rewards. Maintaining social stability through hierarchy can reduce severe conflicts and aggression [171]. Conversely, antagonistic, or aggressive behaviors during FA training, such as those observed in DER rats [151], could interfere with reward learning. Indeed, many reports have correlated social stress with reward learning disruption [172, 173, 174]. The inability to form a stable hierarchical structure could lead to increased competition during reward receipt disrupting normal reward memory association. Food access competition has recently been found to be a fitting marker of the dominance status in rats [175]. In the present study, we analyzed food access order and showed that while each CTR animal maintained a rather stable food access hierarchy, DER rats in groups of three frequently interchanged food access orders on successive FA training days, suggesting that DER animals display elevated competitive behavior. Importantly, many studies have shown that the mPFC [176, 177, 178] and nAC [179] are implicated in determining the hierarchical status of animals during social competition tasks. Collectively, our findings highlight a previously undescribed effect of the DER experience on social stability while they also suggest a neural link between social dynamics and reward prediction-learning which could underlie the behavioral abnormalities observed in DER animals.

4.3. Studying the effects of DER experience on dopaminergic system of reward-related areas

To assess the dopaminergic system status during reward anticipation, D1R levels were measured in nAC and vPFC, two brain regions highly implicated to reward learning and motivated responses [180, 181]. D1R is a G protein – coupled receptor which regulates the function of adenylate cyclase (AC), Ca^{2+} ion channels and therefore, intracellular calcium concentration and neurotransmission [75, 78]. We found that under basal conditions, D1R levels in vPFC were higher in DER adult males, a difference which was not present following FA training. The increased levels of D1R in the vPFC of

DER animals under basal conditions could be a compensatory response to the chronically reduced levels of dopamine in the PFC of DER animals [101]. Contrary to the vPFC, in nAC under basal conditions DER and CTR adult animals displayed similar D1R levels while these levels were increased in the DER group following FA training. According to the literature, the activation of D1R in nAC seems to facilitate social dominance [187]. Consequently, social hierarchy instability caused by elevated general competition could be associated with excessive nAC activation. Taken together, these reports provide a strong argument connecting DER nAC overactivation with increased social instability. Furthermore, a recent study suggested that synergistic mPFC nAC circuit activation encodes behaviors correlated with increased competition such as the approach of an opponent during a competing task [182].

Interestingly, the elevated levels of D1Rs in the PFC of DER animals could confer to their defective contextual-reward association: According to previous studies, the hypothalamic arcuate nucleus (ARC) inhibits dopaminergic activity of VTA in nAC and PFC under food restriction conditions. As a result, the activity of dopaminergic release in VTA is reduced, in order the organism to focus only on food search and not searching of unnecessary rewards in the environment under food restriction conditions [183]. In the case of DER animals, their increased levels of D1Rs could counteract this dopaminergic inhibition and thus show a deficient focus on food reward, and consequently an inefficient context-reward association. If this is the case, DER animals do not make driven exploration for food, but every object in the environment is noticeable for them, a potential source of reward. This could explain why DER animals are hyperactive during cue dependent learning. It is worth noting that hypermobility was detected in humans with obsessive compulsive disorders [184, 185] or in children with ADHD [186].

4.4. Conclusion and future prospects

As a conclusion, this study aimed to elucidate the effects of DER experience on the behavioral output during group food anticipation in adulthood. It was found that DER animals displayed erratic reward learning and elevated unfocused exploration. These effects were accompanied by social instability during food access and alterations of D1Rs in the vPFC. We propose that the stress induced by food restriction together with hierarchical instability led to the context-reward association deficits of DER rats. These effects can reflect pathophysiological characteristics observed in human victims of early

life stress and highlight DER phenotype as a unique early life stress paradigm with distinct phenotypical attributes. By using group behavioral tasks, we simulate more accurately typical human life where everyday challenges include strong social components. Finally, the FA training protocol used in this study proved to be a versatile tool that allowed us to explore many behavioral aspects while also being sufficiently simple and customizable. This study shed light on the neurobiological mechanisms of early life stress that led to social and cognitive impairment in later life. This knowledge will be the introduction of mechanism-based targeted therapies to treat psychological disorders related to child/juvenile trauma, like schizophrenia, major depression, post-traumatic stress disorder (PTSD), attention deficit hyperactivity disorder (ADHD) bipolar disorder, borderline personality [13].

This research investigated the effects of ELS on one aspect of the mesocortical/mesolimbic dopaminergic system and its involvement in reward training in a social context. Apparently, further investigations should be done in order to comprehensively understand the molecular mechanisms behind early life stress effects. Especially, the long-term alterations in dopaminergic system of adult rats, in the case of dopamine transportation and transmission during reward anticipation, and more specifically in the levels of D2R-like dopamine receptors, dopamine transporter (DAT), DARPP-32, need further analysis at the molecular and cellular level. Moreover, using further brain areas, like amygdala and hypothalamus, we may shed more light on the effects of ELS in dopaminergic and reward circuits. Furthermore, investigating the role of social interaction between DER and CTR animals, using mixed post weaning groups, may reveal evidence of reversing the DER phenotype, in adulthood. As a final step, we propose the addition of female rats, apart from male animals, to investigate the factor of sex in the effects of ELS.

Overall, the findings of the present study may open further opportunities for future research, focusing on biomedical knowledge of ELS. This information has substantial translational value for directing clinically feasible strategies to resolve mental illness in adult life.

References

1. Bick, J., & Nelson, C. A. (2015). Early Adverse Experiences and the Developing Brain. *Neuropsychopharmacology, 41*(1), 177–196. doi:10.1038/npp.2015.252
2. Bowlby, J. (1951). Maternal care and mental health. *Bulletin of the World Health Organization, 3, 355–533.*
3. Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies*. Biological Psychiatry, 49*(12)*,* 1023–1039. doi:10.1016/s0006-3223(01)011
4. McEwen, B.S. (2003). Early life influences on life-long patterns of behavior and health. *Mental Retardation and Developmental Disabilities Research Reviews*, *9*(3), 149–154. doi: 10.1002/mrdd.10074.
5. Nemeroff, C. B. (2004). Neurobiological consequences of childhood trauma. *Journal of Clinical Psychiatry*, *65*(Suppl 1), 18–28. *Neuroreport, 16*, 1899–1903.
6. McLaughlin, K. A. (2018). *Early Life Stress and Psychopathology. Oxford Handbooks Online.* doi:10.1093/oxfordhb/9780190681777.013.3
7. B., & Zorrilla Zubilete, M. A. (2016). Long-term effects of early life stress exposure: Role of epigenetic mechanisms. *Pharmacological Research, 109*, 64–73. doi:10.1016/j.phrs.2015.12.033
8. Berczi, I. (2016b). Insights to Neuroimmune Biology. Elsevier.
9. De Bellis, M.D., Baum, A.S., Birmaher, B., Keshavan, M.S., Eccard, C.H., Boring, A.M., Jenkins, F.J., Ryan, N.D. (1999) A.E. Bennett Research Award. Developmental traumatology. Part I: Biological stress systems. Biol Psychiatry 45:1259–1270.
10. Kosten, T.A., Zhang, X.Y., Kehoe, P. (2003). Chronic neonatal isolation stress enhances cocaine-induced increases in ventral striatal dopamine levels in rat pups. *Brain Res Dev Brain Res 141*:109–116.
11. Brake, W.G., Zhang, T.Y., Diorio, J., Meaney, M.J., Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci 19*:1863–1874.
12. Bjorklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. *Trends Neurosci 30*:194–202.
13. Rokita, K. I., Dauvermann, M. R., & Donohoe, G. (2018). Early life experiences and social cognition in major psychiatric disorders: A systematic review. *European Psychiatry, 53*, 123–133. doi:10.1016/j.eurpsy.2018.06.006
14. Stamatakis, A., Diamantopoulou, A., Panagiotaropoulos, T., Raftogianni, A., & Stylianopoulou, F. (2014). A novel model of early experiences involving neonatal learning of a T-maze using maternal contact as a reward or its denial as an event of mild emotional adversity. *Developmental Psychobiology, 56*(8), 1651–1660. doi:10.1002/dev.21248

15. Niwa, M., Matsumoto, Y., Mouri, A., Ozaki, N., and Nabeshima, T. (2011). Vulnerability in early life to changes in the rearing environment plays a crucial role in the aetiopathology of psychiatric disorders. *Int. J. Neuropsychopharmacol. 14*, 459–477. doi: 10.1017/S1461145710001239
16. Sheridan, M. A., McLaughlin, K. A., Winter, W., Fox, N., Zeanah, C., and Nelson, C. A. (2018). Early deprivation disruption of associative learning is a developmental pathway to depression and social problems. *Nat. Commun. 9*, 2216. doi: 10.1038/s41467-018-04381-8
17. Mehta, M. A., Gore-Langton, E., Golembo, N., Colvert, E., Williams, S. C., and Sonuga-Barke, E. (2010). Hyporesponsive reward anticipation in the basal ganglia following severe institutional deprivation early in life*. J. Cogn. Neurosci. 22*, 2316–2325. doi: 10.1162/jocn.2009.21394
18. Goff, B., Gee, D. G., Telzer, E. H., Humphreys, K. L., Gabard-Durnam, L., Flannery, J., et al. (2013). Reduced nucleus accumbens reactivity and adolescent depression following early-life stress. *Neuroscience. 249*, 129–138. doi: 10.1016/j.neuroscience.2012.12.010
19. Hanson, J. L., Hariri, A. R., and Williamson, D. E. (2015). Blunted ventral striatum development in adolescence reflects emotional neglect and predicts depressive symptoms. *Biol Psychiatry. 78*, 598–605. doi: 10.1016/j.biopsych.2015.05.010
20. Kalat, J. K. (2019) *Biological psychology.* Cengage Learning.
21. Diamantopoulou, A., Stamatakis, A., Panagiotaropoulos, T., and Stylianopoulou, F. (2011). Reward or its denial during the neonatal period affects adult spatial memory and hippocampal phosphorylated cAMP response element-binding protein levels of both the neonatal and adult rat. *Neuroscience. 181*, 89–99. doi: 10.1016/j.neuroscience.2011.03.002
22. Champagne, F. A., & Curley, J. P. (2005). How social experiences influence the brain. *Current Opinion in Neurobiology, 15*(6), 704–709. doi:10.1016/j.conb.2005.10.001
23. Levine, S. (1957). Infantile experience and resistance to physiological stress. Science 126, 405
24. Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seck, J. R., Plotsky, P. M. (1996). Early Environmental Regulation of Forebrain Glucocorticoid Receptor Gene Expression: Implications for Adrenocortical Responses to Stress; pp. 49–60. *Developmental Neuroscience, 18*(1-2), 49–60. doi:10.1159/000111395
25. Meaney, M. J., Diorio, J., Francis, D., Weaver, S., Yau, J., Chapman, K., & Seckl, J. R. (2000). Postnatal Handling Increases the Expression of cAMP-Inducible Transcription Factors in the Rat Hippocampus: The Effects of Thyroid Hormones and Serotonin. *The Journal of Neuroscience, 20*(10), 3926–3935. doi:10.1523/jneurosci.20-10-03926.2000
26. Spivey, J. M., Padilla, E., Shumake, J. D., & Gonzalez-Lima, F. (2011). Effects of maternal separation, early handling, and gonadal sex on regional metabolic capacity of the preweanling rat brain. *Brain Research, 1367*, 198–206. doi:10.1016/j.brainres.2010.10.03

27. Pryce, C. R., Bettschen, D., & Feldon, J. (2001). Comparison of the effects of early handling and early deprivation on maternal care in the rat. *Developmental Psychobiology, 38*(4), 239–251. doi:10.1002/dev.1018
28. Denenberg, V. H., Rosenberg, K. M., & Zarrow, M. X. (1969). Mice reared with rat aunts: Effects in adulthood upon plasma corticosterone and open-field activity. *Physiology & Behavior, 4*(5), 705–707. doi:10.1016/0031-9384(69)90104-8
29. Levine, S. (2005). Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology, 30*(10), 939–946. doi:10.1016/j.psyneuen.2005.03.01
30. Meerlo, P., Horvath, K. M., Nagy, G. M., Bohus, B., Koolhaas, J. M. (2001). The Influence of Postnatal Handling on Adult Neuroendocrine and Behavioural Stress Reactivity. *Journal of Neuroendocrinology, 11*(12), 925–933. doi:10.1046/j.1365-2826.1999.00409.x
31. Meaney, M. J., Mitchell, J. B., Aitken, D. H., Bhatnagar, S., Bodnoff, S. R., Iny, L. J., Sarrieau, A. (1991). The effects of neonatal handling on the development of the adrenocortical response to stress: Implications for neuropathology and cognitive deficits in later life. *Psychoneuroendocrinology, 16*(1-3), 85–103. doi:10.1016/0306-4530(91)90072-2
32. Beane, M. L., Cole, M. A., Spencer, R. L., & Rudy, J. W. (2002). Neonatal Handling Enhances Contextual Fear Conditioning and Alters Corticosterone Stress Responses in Young Rats. *Hormones and Behavior, 41*(1), 33–40. doi:10.1006/hbeh.2001.1725
33. De Kloet, E. R., Oitzl, M. S., Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in Neurosciences, 22*(10), 422–426. doi:10.1016/s0166-2236(99)01438-1
34. Escorihuela, R. M., Tobeña, A., & Fernández-Teruel, A. (1994). Environmental enrichment reverses the detrimental action of early inconsistent stimulation and increases the beneficial effects of postnatal handling on shuttlebox learning in adult rats. *Behavioural Brain Research, 61*(2), 169–173. doi:10.1016/0166-4328(94)90157-0
35. Vallée, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari, S. (1997). Prenatal Stress Induces High Anxiety and Postnatal Handling Induces Low Anxiety in Adult Offspring: Correlation with Stress-Induced Corticosterone Secretion. *The Journal of Neuroscience, 17*(7), 2626–2636. doi:10.1523/jneurosci.17-07-02626.1997
36. Durand, M., Sarrieau, A., Aguerre, S., Mormède, P., Chaouloff, F. (1998). Differential effects of neonatal handling on anxiety, corticosterone response to stress, and hippocampal glycocorticoid and serotonin (5-HT)2 receptors in lewis rats. *Psychoneuroendocrinology, 23*(4), 323–335. doi:10.1016/s0306-4530(98)00011-0
37. Tejedor-Real, P., Costela, C., & Gibert-Rahola, J. (1997). Neonatal handling reduces emotional reactivity and susceptibility to learned helplessness. Involvement of catecholaminergic systems. *Life Sciences, 62*(1), 37–50. doi:10.1016/s0024-3205(97)01036-9

38. Kosten, T. A., Lee, H. J., & Kim, J. J. (2007). Neonatal handling alters learning in adult male and female rats in a task-specific manner. *Brain Research, 1154*, 144–153. doi:10.1016/j.brainres.2007.03.08
39. Todeschin, A. S., Winkelmann-Duarte, E. C., Jacob, M. H. V., Aranda, B. C. C., Jacobs, S., Fernandes, M. C., Ribeiro, M. F., Sanvitto, G. L., Lucion, A. B. (2009). Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior, 56*(1), 93–100. doi:10.1016/j.yhbeh.2009.03.006
40. Padoin, M. J., Cadore, L. P., Gomes, C. M., Barros, H. M., Lucion, A. B. (2001). Long-lasting effects of neonatal stimulation on the behavior of rats. *Behav Neurosci., 115*(6):1332-40. doi: 10.1037//0735-7044.115.6.1332
41. Aguilar, R., Caramés, J. M., & Espinet, A. (2009). Effects of neonatal handling on playfulness by means of reversal of the desire to play in rats (Rattus norvegicus). *Journal of Comparative Psychology, 123*(4), 347–356. doi:10.1037/a001643
42. Liu, Caldji, Sharma, Plotsky, & Meaney. (2008). Influence of Neonatal Rearing Conditions on Stress-Induced Adrenocorticotropin Responses and Norepinepherine Release in the Hypothalamic Paraventricular Nucleus*. Journal of Neuroendocrinology, 12*(1), 5–12. doi:10.1046/j.1365-2826.2000.00422.x
43. Azevedo, M. S., de Souza, F. L., Donadio, M. V. F., Lucion, A. B., & Giovenardi, M. (2010). Interventions in the neonatal environment in rats and their relationship to behavior in adulthood and maternal behavior. *Psychology & Neuroscience, 3(*1), 73–78.
44. Lehmann, J., & Feldon, J. (2000). Long-term Biobehavioral Effects of Maternal Separation in the Rat: Consistent or Confusing? *Reviews in the Neurosciences, 11*(4). doi:10.1515/revneuro.2000.11.4.38
45. Rosenfeld, P., Wetmore, J. B., & Levine, S. (1992). Effects of repeated maternal separations on the adrenocortical response to stress of preweaning rats*. Physiology & Behavior, 52*(4), 787–791. doi:10.1016/0031-9384(92)90415-x
46. Rosenfeld, P., van Eekelen, J. A., Levine, S., de Kloet, E. R. (1993). Ontogeny of corticosteroid receptors in the brain. *Cellular and Molecular Neurobiology, 13*,295-319
47. Lévy, F., Melo, A. I., Galef, B. G., Madden, M., & Fleming, A. S. (2003). Complete maternal deprivation affects social, but not spatial, learning in adult rats. *Developmental Psychobiology, 43*(3), 177–191. doi:10.1002/dev.10131
48. Garner, B., Wood, S., Pantelis, C., & Vandenbuuse, M. (2007). Early maternal deprivation reduces prepulse inhibition and impairs spatial learning ability in adulthood: No further effect of post-pubertal chronic corticosterone treatment. Behavioural Brain Research, 176(2), 323–332. doi:10.1016/j.bbr.2006.10.020
49. Dent, G. W., Okimoto, D. K., Smith, M. A., & Levine, S. (2000). Stress-Induced Alterations in Corticotropin-Releasing Hormone and Vasopressin Gene Expression in the Paraventricular Nucleus during Ontogeny. *Neuroendocrinology, 71*(6), 333–342. doi:10.1159/000054554

50. Poleksic, J., Aksic, M., Kapor, S., Aleksic, D., Stojkovic, T., Radovic, M., Djulejic, V., Markovic, B., Stamatakis, A. (2021). Effects of Maternal Deprivation on the Prefrontal Cortex of Male Rats: Cellular, Neurochemical, and Behavioral Outcomes. *Front. Behav. Neurosci. 15*:666547. doi: 10.3389/fnbeh.2021.666547
51. Aisa, B., Tordera, R., Lasheras, B., Del Río, J., Ramírez, M. J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology, 32*(3) 256-266. doi:10.1016/j.psyneuen.2006.12.013
52. Koehl, M., Darnaudéry, M., Dulluc, J., Van Reeth, O., Moal, M.L. and Maccari, S. (1999), Prenatal stress alters circadian activity of hypothalamo–pituitary–adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. *J. Neurobiol., 40*: 302-315. https://doi.org/10.1002/(SICI)1097-4695(19990905)40:3<302::AID-NEU3>3.0.CO;2-7
53. Panagiotaropoulos, T., Diamantopoulou, A., Stamatakis, A., Dimitropoulou, M., & Stylianopoulou, F. (2009). Learning of a T-maze by rat pups when contact with the mother is either permitted or denied. *Neurobiology of Learning and Memory, 91*, 2–12.
54. Diamantopoulou, A., Raftogianni, A., Stamatakis, A., Tzanoulinou, S., Oitzl, M. S., & Stylianopoulou, F. (2013). Denial or receipt of expected reward through maternal contact during the neonatal period differentially affect the development of the rat amygdala and program its function in adulthood in a sex-dimorphic way. *Psychoneuroendocrinology, 38*, 1757–1771.
55. Conely L., & Bell, R. W. (1978). Neonatal ultrasounds elicited by odor cues. *Developmental Psychobiology, 11*, 193–197.
56. D'Amato F. R., & Cabib, S. (1987). Chronic exposure to a novel odor increases pups' vocalizations, maternal care, and alters dopaminergic functioning in developing mice*. Behavioral Neural Biology, 48*, 197–205.
57. Lyons D. M., & Banks, E. M. (1982). Ultrasounds in neonatal rats: Novel, predator and conspecific odor cues. *Developmental Psychobiology, 15*, 455–46
58. Stamatakis, A., Manatos, V., Kalpachidou, T., and Stylianopoulou, F. (2016). Exposure to a mildly aversive early life experience leads to prefrontal cortex deficits in the rat. *Brain Struct. Funct. 221*, 4141–4157. doi: 10.1007/s00429-015-1154-0
59. Stamatakis, A., Diamantopoulou, A., Panagiotaropoulos, T., Raftogianni, A., & Stylianopoulou, F. (2014). A novel model of early experiences involving neonatal learning of a T-maze using maternal contact as a reward or its denial as an event of mild emotional adversity. *Developmental Psychobiology, 56(*8), 1651–1660. doi:10.1002/dev.21248
60. Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan as Reserpine Antagonists. *Nature, 180*(4596), 1200–1200. doi:10.1038/1801200a0
61. Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. *Zeitschrift für Zellforschung Und Mikroskopische Anatomie, 65*(4), 573–596. doi:10.1007/bf00337069

62. Nieoullon, A., & Coquerel, A. (2003). Dopamine. *Current Opinion in Neurology, 16*, S3–S9. doi:10.1097/00019052-200312002-00002
63. Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience, 5*(6), 483–494. doi:10.1038/nrn1406
64. Salamone, J., Correa, M., Mingote, S., & Weber, S. (2005). Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Current Opinion in Pharmacology, 5*(1), 34–41. doi:10.1016/j.coph.2004.09.004
65. Berridge, K. C. (2006). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology, 191*(3), 391–431. doi:10.1007/s00213-006-0578-x.
66. Silberman, D. M., Acosta, G. B., & Zorrilla Zubilete, M. A. (2016). Long-term effects of early life stress exposure: Role of epigenetic mechanisms. *Pharmacological Research, 109*, 64–73. doi:10.1016/j.phrs.2015.12.033
67. Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Pharmacological Reviews, 63*(1), 182–217. doi:10.1124/pr.110.002642.
68. Yanowitch, R., & Coccaro, E. F. (2011). The Neurochemistry of Human Aggression. *Aggression*, 151–169. doi:10.1016/b978-0-12-380858-5.00005-8.
69. Tritsch, N. X., & Sabatini, B. L. (2012). Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. *Neuron, 76*(1), 33–50. doi:10.1016/j.neuron.2012.09.023
70. Andén, N.-E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N.-Å., & Larsson, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sciences, 3*(6), 523–530. doi:10.1016/0024-3205(64)90161-4
71. Miller, B. L., & Cummings, J. L. (Eds.). (2007). The human frontal lobes: Functions and disorders (2nd ed.). The Guilford Press
72. Albanese, A., Altavista, M. C., Rossi, P. (1986). Organization of central nervous system dopaminergic pathways. *Journal of Neural transmission. Supplementum, 22*:3–17. PMID: 346587
73. Xu, H., Yang, F. (2022). The interplay of dopamine metabolism abnormalities and mitochondrial defects in the pathogenesis of schizophrenia. *Transl Psychiatry 12*, 464. https://doi.org/10.1038/s41398-022-02233-0
74. Baik, J. H. (2013). Dopamine signaling in reward-related behaviors. *Front. Neural Circ. 7*, 152. doi: 10.3389/fncir.2013.00152
75. Kim A. Neve, Jeremy K. Seamans & Heather Trantham-Davidson (2004). Dopamine Receptor Signaling, *Journal of Receptors and Signal Transduction, 24*:3, 165-205, DOI:10.1081/RRS-200029981
76. Hall, F. S., Wilkinson, L. S., Humby, T., & Robbins, T. W. (1999). Maternal deprivation of neonatal rats produces enduring changes in dopamine function. *Synapse, 32*(1), 37–43. doi:10.1002/(sici)1098-2396(199904)32:1<37::aid-syn5>3.0.co;2-4

77. Knab, A.M., Lightfoot, J.T. (2010). Does the difference between physically active and couch potato lie in the dopamine system*? Int J Biol Sci, 6*(2):133-150. doi:10.7150/ijbs.6.133. https://www.ijbs.com/v06p0133.htm
78. Bhatia, A., Lenchner, J.R., Saadabadi, A. (2020). Biochemistry, Dopamine Receptors. *StatPearls*
79. Hikida, T., Kimura, K., Wada, N., Funabiki, K., & Nakanishi, S. (2010). Distinct Roles of Synaptic Transmission in Direct and Indirect Striatal Pathways to Reward and Aversive Behavior. *Neuron, 66*(6), 896–907. doi:10.1016/j.neuron.2010.05.011
80. Hikida, T., Yawata, S., Yamaguchi, T., Danjo, T., Sasaoka, T., Wang, Y., & Nakanishi, S. (2012). Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors. *Proceedings of the National Academy of Sciences, 110*(1), 342–347. doi:10.1073/pnas.1220358110
81. Xiu, J., Zhang, Q., Zhou, T., Zhou, T., Chen, Y., & Hu, H. (2014). Visualizing an emotional valence map in the limbic forebrain by TAI-FISH. *Nature Neuroscience, 17*(11), 1552–1559. doi:10.1038/nn.3813
82. Pryce, C. R., Rüedi-Bettschen, D., Dettling, A. C., Weston, A., Russig, H., Ferger, B., & Feldon, J. (2005). Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. *Neuroscience & Biobehavioral Reviews, 29*(4-5), 649–674. doi:10.1016/j.neubiorev.2005.03.0
83. Champagne, F. A., & Curley, J. P. (2009). Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience & Biobehavioral Reviews, 33*(4), 593–600. doi:10.1016/j.neubiorev.2007.10.
84. Schmid, B., Blomeyer, D., Buchmann, A. F., Trautmann-Villalba, P., Zimmermann, U. S., Schmidt, M. H., Esser, G., Banaschewski, T., Laucht, M. (2011). Quality of early mother–child interaction associated with depressive psychopathology in the offspring: A prospective study from infancy to adulthood. *Journal of Psychiatric Research, 45*(10), 1387–1394. doi:10.1016/j.jpsychires.2011.05
85. Matthews, K., Dalley, J. W., Matthews, C., Hu Tsai, T., & Robbins, T. W. (2001). Periodic maternal separation of neonatal rats produces region- and gender-specific effects on biogenic amine content in postmortem adult brain. *Synapse, 40*(1), 1–10. doi:10.1002/1098-2396(200104)40:1<1::aid-syn1020>3.0.co;2-e
86. Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences, 30*(5), 194–202. doi:10.1016/j.tins.2007.03.006
87. Rodrigues, A.-J., Leão, P., Carvalho, M., Almeida, O. F. X., & Sousa, N. (2010). Potential programming of dopaminergic circuits by early life stress. *Psychopharmacology, 214*(1), 107–120. doi:10.1007/s00213-010-2085-3

88. Lovic, V., Belay, H., Walker, C.-D., Burton, C. L., Meaney, M. J., Sokolowski, M., & Fleming, A. S. (2013). Early postnatal experience and DRD2 genotype affect dopamine receptor expression in the rat ventral striatum. *Behavioural Brain Research*, 237, 278–282. doi:10.1016/j.bbr.2012.09.046
89. Papaioannou, A., Dafni, U., Alikaridis, F., Bolaris, S., & Stylianopoulou, F. (2002). Effects of neonatal handling on basal and stress-induced monoamine levels in the male and female rat brain. *Neuroscience, 114*(1), 195–206. doi:10.1016/s0306-4522(02)00129-x
90. Gariépy, J.-L., Rodriguiz, R. M., & Jones, B. C. (2002). Handling, genetic and housing effects on the mouse stress system, dopamine function, and behavior. *Pharmacology Biochemistry and Behavior, 73*(1), 7–17. doi:10.1016/s0091-3057(02)00789-x
91. Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology, 27*(1-2), 127–138. doi:10.1016/s0306-4530(01)00040-3
92. Lejeune, S., Dourmap, N., Martres, M.-P., Giros, B., Daugé, V., & Naudon, L. (2013). The dopamine D1 receptor agonist SKF 38393 improves temporal order memory performance in maternally deprived rats. *Neurobiology of Learning and Memory*, *106*, 268–273. doi:10.1016/j.nlm.2013.10.005
93. Rentesi, G., Antoniou, K., Marselos, M., Syrrou, M., Papadopoulou-Daifoti, Z., & Konstandi, M. (2013). Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. *Behavioural Brain Research*, *244*, 29–37. doi:10.1016/j.bbr.2013.01.040
94. Zhang, T. Y. (2005). Influence of Naturally Occurring Variations in Maternal Care on Prepulse Inhibition of Acoustic Startle and the Medial Prefrontal Cortical Dopamine Response to Stress in Adult Rats. *Journal of Neuroscience, 25*(6), 1493–1502. doi:10.1523/jneurosci.3293-04.2005
95. Ventura, R., Coccurello, R., Andolina, D., Latagliata, E. C., Zanettini, C., Lampis, V., Battaglia, M., D'Amato, F., R., Moles, A. (2012). Postnatal Aversive Experience Impairs Sensitivity to Natural Rewards and Increases Susceptibility to Negative Events in Adult Life. *Cerebral Cortex, 23*(7), 1606–1617. doi:10.1093/cercor/bhs145
96. Lewis, R.G., Florio, E., Punzo, D., Borrelli, E. (2021). The Brain's Reward System in Health and Disease. In: Engmann, O., Brancaccio, M. (eds) Circadian Clock in Brain Health and Disease. *Advances in Experimental Medicine and Biology*, vol 1344. Springer, Cham. https://doi.org/10.1007/978-3-030-81147-1_4
97. Schultz, W. (2015). Neuronal Reward and Decision Signals: From Theories to Data. *Physiological Reviews, 95*(3), *853–951.* doi:10.1152/physrev.00023.2014
98. Kolb, B., & Whishaw, I. Q. (2001). An introduction to brain and behavior. Worth Publishers.
99. Berridge, K. C., Kringelbach, M. L. (2015). Pleasure Systems in the Brain. *Neuron, 86*(3), 646–664. doi:10.1016/j.neuron.2015.02.018
100. Berridge, K. C., Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences, 26*(9), 507–513. doi:10.1016/s0166-2236(03)00233-9

101. Raftogianni, A., Stamatakis, A., Diamantopoulou, A., Kollia, A.-M., & Stylianopoulou, F. (2014). Effects of an early experience of reward through maternal contact or its denial on the dopaminergic system of the rat brain. *Neuroscience, 269*, 11–20. doi:10.1016/j.neuroscience.2014.0
102. Jackson, M. E., Frost, A. S., & Moghaddam, B. (2001). Stimulation of prefrontal cortex at physiologically relevant frequencies inhibits dopamine release in the nucleus accumbens. *Journal of Neurochemistry, 78*(4), 920–923. doi:10.1046/j.1471-4159.2001.00499.x
103. Del Arco, A., & Mora, F. (2008). Prefrontal cortex–nucleus accumbens interaction: In vivo modulation by dopamine and glutamate in the prefrontal cortex. *Pharmacology Biochemistry and Behavior, 90*(2), 226–235. doi:10.1016/j.pbb.2008.04.011
104. Baik, J.-H. (2013). Dopamine Signaling in reward-related behaviors. Frontiers in Neural Circuits, 7. doi:10.3389/fncir.2013.00152
105. Wyvell, C. L., & Berridge, K. C. (2000). Intra-Accumbens Amphetamine Increases the Conditioned Incentive Salience of Sucrose Reward: Enhancement of Reward "Wanting" without Enhanced "Liking" or Response Reinforcement. The Journal of Neuroscience, 20(21), 8122–8130. doi:10.1523/jneurosci.20-21-08122.2000
106. Berridge, K. C. (2006). The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology, 191(3), 391–431. doi:10.1007/s00213-006-0578-x
107. Haber, S. N., & Knutson, B. (2009). The Reward Circuit: Linking Primate Anatomy and Human Imaging. *Neuropsychopharmacology, 35*(1), 4–26. doi:10.1038/npp.2009.12
108. Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences, 13*, 177–184. doi:10.1016/0165-6147(92)90060-j
109. Wise, R. A., & Hoffman, D. C. (1992). Localization of drug reward mechanisms by intracranial injections. *Synapse, 10*(3), 247–263. doi:10.1002/syn.890100307
110. Di Chiara, G. (1995). The role of dopamine in drug abuse viewed from the perspective of its role in motivation. *Drug and Alcohol Dependence, 38(*2), 95–137. doi:10.1016/0376-8716(95)01118-i
111. Wise, R. A. (2008). Dopamine and reward: The anhedonia hypothesis 30 years on. *Neurotoxicity Research, 14*(2-3), 169–183. doi:10.1007/bf03033808
112. Schultz, W., Dayan, P., & Montague, P. R. (1997). A Neural Substrate of Prediction and Reward. *Science, 275*(5306), 1593–1599. doi:10.1126/science.275.5306.1593
113. Palmiter, R. D. (2007). Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci. 30*, 375–381. doi: 10.1016/j.tins.2007.06.004
114. Sheridan, M. A., McLaughlin, K. A., Winter, W., Fox, N., Zeanah, C., & Nelson, C. A. (2018). Early deprivation disruption of associative learning is a developmental pathway to depression and social problems. *Nature Communications, 9*(1). doi:10.1038/s41467-018-04381-8

115. Mehta, M. A., Gore-Langton, E., Golembo, N., Colvert, E., Williams, S. C., and Sonuga-Barke, E. (2010). Hyporesponsive reward anticipation in the basal ganglia following severe institutional deprivation early in life*. J. Cogn. Neurosci. 22*, 2316–2325.doi: 10.1162/jocn.2009.21394

116. Goff, B., Gee, D. G., Telzer, E. H., Humphreys, K. L., Gabard-Durnam, L., Flannery, J., et al. (2013). Reduced nucleus accumbens reactivity and adolescent depression following early-life stress. *Neuroscience. 249*, 129–138. doi: 10.1016/j.neuroscience.2012.12.010

117. Hanson, J. L., Hariri, A. R., and Williamson, D. E. (2015). Blunted ventral striatum development in adolescence reflects emotional neglect and predicts depressive symptoms. *Biol Psychiatry. 78*, 598–605. doi: 10.1016/j.biopsych.2015.05.010

118. Ploj, K., and Nylander, I. (2003). Long-term effects on brain opioid and opioid receptor like-1 receptors after short periods of maternal separation in rats. *Neurosci. Lett. 345*, 195–197. doi: 10.1016/S0304-3940(03)00515-9

119. Callaghan, B. L., and Richardson, R. (2013). Early experiences and the development of emotional learning systems in rats. *Biol. Mood Anx. Disord. 3*, 8. doi: 10.1186/2045-5380-3-8

120. Payer, D., Williams, B., Mansouri, E., Stevanovski, S., Nakajima, S., Le Foll, B., Kish, S., Houle, S., Mizrahi, R., George, S. R., George, T. P., Boileau, I. (2017). Corticotropin-releasing hormone and dopamine release in healthy individuals. *Psychoneuroendocrinology, 76*, 192–196. doi:10.1016/j.psyneuen.2016.11.034

121. Refojo, D., Schweizer, M., Kuehne, C., Ehrenberg, S., Thoeringer, C., Vogl, A. M., … Deussing, J. M. (2011). Glutamatergic and Dopaminergic Neurons Mediate Anxiogenic and Anxiolytic Effects of CRHR1. *Science, 333*(6051), 1903–1907. doi:10.1126/science.1202107

122. Russo, S. J., & Nestler, E. J. (2013). The brain reward circuitry in mood disorders. *Nature Reviews Neuroscience, 14*(9), 609–625. doi:10.1038/nrn3381

123. Miller, E.K., Freedman, D.J., Wallis, J.D. (2002). The prefrontal cortex: categories, concepts and cognition. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 357*(1424): 1123–1136. doi:10.1098/rstb.2002.1099.

124. Diamond, A. (2002). Normal Development of Prefrontal Cortex from Birth to Young Adulthood: Cognitive Functions, Anatomy, and Biochemistry, in Donald T. Stuss, and Robert T. Knight (eds), *Principles of Frontal Lobe* Function (pp. 466-503). New York, NY: Oxford University Press*.* https://doi.org/10.1093/acprof:oso/9780195134971.003.0029

125. Kolb, B., & Nonneman, A. (1976). Functional development of prefrontal cortex in rats continues into adolescence. *Science, 193*(4250), 335–336. doi:10.1126/science.935872

126. Price, J. L. (1999). Prefrontal Cortical Networks Related to Visceral Function and Mood. Annals of the New York *Academy of Sciences, 877*(1), 383–396. doi:10.1111/j.1749-6632.1999.tb09278.x

127. Burgos-Robles, A., Bravo-Rivera, H., & Quirk, G. J. (2013). Prelimbic and Infralimbic Neurons Signal Distinct Aspects of Appetitive Instrumental Behavior. *PLoS ONE, 8(*2), e57575. doi:10.1371/journal.pone.0057575

128. Hare, T. A., Camerer, C. F., Rangel, A. (2009) Self-control in decision-making involves modulation of the vmPFC valuation system. *Science 324*(5927) 646–648. doi:10.1126/science.1168450

129. Bartra, O., McGuire, J. T., Kable, J. W. (2013). The valuation system: a coordinate-based meta-analysis of BOLD fMRI experiments examining neural correlates of subjective value. *NeuroImage (76*)412-427. https://doi.org/10.1016/j.neuroimage.2013.02.06

130. Clark, L., Cools, R., & Robbins, T. W. (2004). The neuropsychology of ventral prefrontal cortex: Decision-making and reversal learning. *Brain and Cognition, 55*(1), 41–53. doi:10.1016/s0278-2626(03)00284-7

131. Aron, A. R., Robbins, T. W., & Poldrack, R. A. (2004). Inhibition and the right inferior frontal cortex. *Trends in Cognitive Sciences, 8*(4), 170–177. doi:10.1016/j.tics.2004.02.010

132. Segal, O., Elkana, O. (2023). The ventrolateral prefrontal cortex is part of the modular working memory system: A functional neuroanatomical perspective*. Front. Neuroanat. 17*:1076095. doi: 10.3389/fnana.2023.1076095

133. Kohn, N., Eickhoff, S. B., Scheller, M., Laird, A. R., Fox, P. T., & Habel, U. (2014). Neural network of cognitive emotion regulation — An ALE meta-analysis and MACM analysis. *NeuroImage, 87*, 345–355. doi:10.1016/j.neuroimage.2013.11.

134. Yin, H. H., Ostlund, S. B., Balleine, B. W. (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *The European Journal of Neuroscience, 28*(8): 1437–48. doi:10.1111/j.1460-9568.2008.06422.x. PMC 2756656. PMID 18793321.

135. Stamatakis, A., Manatos, V., Kalpachidou, T., & Stylianopoulou, F. (2015). Exposure to a mildly aversive early life experience leads to prefrontal cortex deficits in the rat. *Brain Structure and Function, 221*(8), 4141–4157. doi:10.1007/s00429-015-1154-0

136. Goff, B., Gee, D. G., Telzer, E. H., Humphreys, K. L., Gabard-Durnam, L., Flannery, J., & Tottenham, N. (2013). Reduced nucleus accumbens reactivity and adolescent depression following early-life stress. *Neuroscience, 249*, 129–138. doi:10.1016/j.neuroscience.2012.12.010

137. Čater, M., & Majdič, G. (2021). How early maternal deprivation changes the brain and behavior? *European Journal of Neuroscience*. doi.org/10.1111/ejn.15238

138. Bick, J., & Nelson, C. A. (2015). Early Adverse Experiences and the Developing Brain. *Neuropsychopharmacology, 41*(1), 177–196. doi:10.1038/npp.2015.252

139. Lemos, C., Salti, A., Amaral, I. M., Fontebasso, V., Singewald, N., Dechant, G., Hofer, A., El Rawas, R. (2021). Social interaction reward in rats has anti-stress effects. *Addict. Biol. 26*, doi: 10.1111/adb.12878

140. Lucas, G. A., Timberlake, W., and Gawley, D. J. (1988). Adjunctive behavior of the rat under periodic food delivery in a 24-h environment. *Anim. Learn. Behav. 16*, 19–30, doi: 10.3758/BF03209039

141. Patton,D. F., Katsuyama, A.M., Pavlovski, I.,Michalik,M., Patterson, Z., Parfyonov, M., et al. (2014). Circadian mechanisms of food anticipatory rhythms in rats fed once or twice daily: clock gene and endocrine correlates. *PloS one. 9*, doi: 10.1371/journal.pone.0112451

142. Dietze, S., Lees, K. R., Fink, H., Brosda, J., and Voigt, J. P. (2016). Food deprivation, body weight loss and anxiety-related behavior in rats. *Animals (Basel). 6*, doi: 10.3390/ani6010004

143. Paxinos, G., and Watson, C. (2014). Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, 7th Edition. Elsevier Academic Press, San Diego.

144. Griem-Krey, N., Klein, A. B., Herth, M., & Wellendorph, P. (2019). Autoradiography as a Simple and Powerful Method for Visualization and Characterization of Pharmacological Targets. *Journal of Visualized Experiments*, (145). doi:10.3791/58879

145. Bruce, R. H. (1941). An experimental analysis of social factors affecting the performance of white rats. III. Dominance and cooperation motivated by water and food deprivation. *J. Comp. Psychol. 31*, 395-412 doi: 10.1037/h0056901

146. Gould, T. D., Dao, D. T., and Kovacsics, C. E. (2009). The open field test in Mood and Anxiety Related Phenotypes in Mice. Neuromethods, eds Gould, T. (Totowa, NJ: Humana Press).

147. Corey, D. (1978). T. The determinants of exploration and neophobia. *Neurosci. Biobehav. Rev. 2*, 235–253. doi: 10.1016/0149-7634(78)90033-7

148. Meaney,M. J., Tannenbaum, B., Francis,D., Bhatnagar, S., Shanks,N., Viau, V., et al. (1994). Early environmental programming hypothalamic-pituitary-adrenal responses to stress. Semin. *Neurosci. 6*, 247–259. doi: 10.1006/smns.1994.1032

149. Rygula, R., Abumaria,N., Flügge, G., Fuchs, E., Rüther, E., andHavemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behav. Brain Res. 162*, 127–134. doi: 10.1016/j.bbr.2005.03.009

150. Diamantopoulou, A., Kalpachidou, T., Aspiotis, G., Gampierakis, I., Stylianopoulou, F., and Stamatakis, A. (2018). An early experience of mild adversity involving temporary denial of maternal contact affects the serotonergic system of adult male rats and leads to a depressive-like phenotype and inability to adapt to a chronic social stress. *Physiol. Behav. 184*, 46–54. doi: 10.1016/j.physbeh.2017.11.004

151. Diamantopoulou, A., Raftogianni, A., Stamatakis, A., Alikaridis, F., Oitzl, M. S., and Stylianopoulou, F. (2012). Denial of reward in the neonate shapes sociability and serotonergic activity in the adult rat. *PLoS One. 7*. doi: 10.1371/journal.pone.0033793

152. Heiderstadt, K. M., McLaughlin, R. M., Wright, D. C., Walker, S. E., and Gomez-Sanchez, C. E. (2000). The effect of chronic food and water restriction on openfield behaviour and serum corticosterone levels in rats. *Lab. Anim. 34*, 20–28. doi: 10.1258/002367700780578028

153. Tomiyama, A. J., Mann, T., Vinas, D., Hunger, J. M., Dejager, J., and Taylor, S. E. (2010). Low calorie dieting increases cortisol. *Psychosom. Med. 72*, 357–364. doi: 10.1097/PSY.0b013e3181d9523c

154. Carnevali, L., Montano, N., Statello, R., Coud,é, G., Vacondio, F., Rivara, S., et al. (2017). Social stress contagion in rats: behavioural, autonomic and neuroendocrine correlates. *Psychoneuroendocrinology. 82*, 155–163. doi: 10.1016/j.psyneuen.2017.05.017

155. Carnevali, L., Montano, N., Tobaldini, E., Thayer, J. F., and Sgoifo, A. (2020). The contagion of social defeat stress: Insights from rodent studies. *Neurosci. Biobehav. Rev. 111*, 12–18. doi: 10.1016/j.neubiorev.2020.01.011

156. Murphy, J. M., and Nagy, Z. M. (1979). FLA-63 blocks food-deprivation-induced behavioral arousal in the mouse. *Psychobiology 7*, 407–411. doi: 10.3758/BF03326666

157. Timberlake, W., and White, W. (1990). Winning isn't everything: Rats need only food deprivation and not food reward to efficiently traverse a radial arm maze. *Learn. Motiv. 21*, 153–163. doi: 10.1016/0023-9690(90)90017-I

158. Kosten, T. A., Kim, J. J., and Lee, H. J. (2012). Early life manipulations alter learning and memory in rats. *Neurosci. Biobehav. Rev. 36*, 1985–2006. doi: 10.1016/j.neubiorev.2012.07.003

159. Loi, M., Sarabdjitsingh, R. A., Tsouli, A., Trinh, S., Arp, M., Krugers, H. J., Karst, H., van den Bos, R., Joëls, M. (2017). Transient prepubertal mifepristone treatment normalizes deficits in contextual memory and neuronal activity of adult male rats exposed to maternal deprivation. *eNeuro. 4*, 5. doi: 10.1523/ENEURO.0253-17.2017

160. Cléry-Melin, M., Jollant, F., and Gorwood, P. (2019). Reward systems and cognitions in Major Depressive Disorder. *CNS Spectrums. 24*, 64–77. doi: 10.1017/S1092852918001335

161. Goldfarb, E. V., Tompary, A., Davachi, L., and Phelps, E. A. (2019). Acute stress throughout the memory cycle: Diverging effects on associative and item memory. *J. Exp. Psychol. Gen. 148*, 13–29. doi: 10.1037/xge0000472

162. De Quervain, D. J., Roozendaal, B., and McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature. 394*, 787–790. doi: 10.1038/29542

163. Sunanda, B. S., Shankaranarayana, R., and Raju, T. R. (2000). Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. *Curr. Sci. 79*, 1581–1584.

164. Moreira, P. S., Almeida, P. R., Leite-Almeida, H., Sousa, N., and Costa, P. (2016). Impact of chronic stress protocols in learning and memory in rodents: systematic review and meta-analysis. *PLoS One. 11*. doi: 10.1371/journal.pone.0163245

165. Hodge, S. J., Thornton, A., Flower, T. P., and Clutton-Brock, T. H. (2009). Food limitation increases aggression in juvenile meerkats. *Behav. Ecol. 20*, 930–935. doi: 10.1093/beheco/arp071

166. Fitzpatrick, C. J., and Morrow, J. D. (2016). Pavlovian conditioned approach training in rats. *J. Vis. Exp. 108*. doi: 10.3791/53580

167. Abrahamsen, G. C., and Carr, K. D. (1996). Effects of corticosteroid synthesis inhibitors on the sensitization of reward by food restriction. *Brain Res. 726*, 39–48. doi: 10.1016/0006-8993(96)00289-2

168. Tapp, D. N., Zerkle, H. L., and McMurray, M. S. (2020). Extent of food restriction affects probability but not delay-based decision-making. *Jrnl Exper Anal. Behav. 114*, 179–192. doi: 10.1002/jeab.624

169. Hill, R. A., Klug, M., Kiss Von Soly, S., Binder, M. D., Hannan, A. J., van den Buuse, M. (2014). Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus. 24*, 1197–1211. doi: 10.1002/hipo. 22302

170. Schwabe, L., Schächinger,H., de Kloet, E. R., and Oitzl,M. S. (2010). Corticosteroids operate as a switch between memory systems. *J. Cogn. Neurosci. 22*, 1362–1372. doi: 10.1162/jocn.2009.21278

171. Curley, J. P. (2016). Temporal pairwise-correlation analysis provides empirical support for attention hierarchies in mice. *Biol. Lett. 12*, 0192. doi: 10.1098/rsbl.2016.0192

172. van der Harst, J. E., Baars, A. M., and Spruijt, B. M. (2005). Announced rewards counteract the impairment of anticipatory behaviour in socially stressed rats. *Behav Brain Res. 161*, 183–189. doi: 10.1016/j.bbr.2005.02.029

173. Kamal, A., Van der Harst, J. E., Kapteijn, C. M., Baars, A. J., Spruijt, B. M., and Ramakers, G. M. (2010). Announced reward counteracts the effects of chronic social stress on anticipatory behavior and hippocampal synaptic plasticity in rats. *Exp. Brain Res. 201*, 641–651. doi: 10.1007/s00221-009-2083-z

174. Kúkel'ov,á D., Bergamini, G., Sigrist, H., Seifritz, E., Hengerer, B., and Pryce, C. R. (2018). Chronic social stress leads to reduced gustatory reward salience and effort valuation in mice. Front. *Behav. Neurosci. 12*, 134. doi: 10.3389/fnbeh.2018.00134

175. Costa, D. F., Moita, M. A., and Márquez, C. (2021). Novel competition test for food rewards reveals stable dominance status in adult male rats. *Sci. Rep. 11*, 14599. doi: 10.1038/s41598-021-93818-0

176. Benner, S., Endo, T., Endo, N., Kakeyama, M., and Tohyama, C. (2014). Early deprivation induces competitive subordinance in C57BL/6 male mice. *Physiol. Behav. 137*, 42–52. doi: 10.1016/j.physbeh.2014.06.018

177. Zhou, T., Zhu, H., Fan, Z., Wang, F., Chen, Y., Liang, H., et al. (2017). History of winning remodels thalamo-PFC circuit to reinforce social dominance. *Science. 357*, 162–168. doi: 10.1126/science.aak9726

178. Padilla-Coreano, N., Batra, K., and Patarino, M. (2022). Cortical ensembles orchestrate social competition through hypothalamic outputs. *Nature. 603*, 667–671. doi: 10.1038/s41586-022-04507-5

179. Shan, Q., Hu, Y., Chen, S., and Tian, Y. (2022). Nucleus accumbens dichotomically controls social dominance in male mice. *Neuropsychopharmacology. 47*, 776–787. doi: 10.1038/s41386-021-01220-1

180. Salamone, J. D., and Correa, M. (2002). Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav. Brain Res. 137*, 3–25. doi: 10.1016/S0166-4328(02)00282-6

181. Hiser, J., and Koenigs, M. (2018). The multifaceted role of the ventromedial prefrontal cortex in emotion, decision making, social cognition, and psychopathology. *Biol. Psychiatr. 83*, 638–647. doi: 10.1016/j.biopsych.2017. 10.030

182. Van Der Kooij, M. A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., Grosse, J., Guillot de Suduiraut, I, Canto, C., Sandi, C. (2018). Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. *Mol. Psychiatr. 23*, 569–578. doi: 10.1038/mp.2017.135

183. Tae-Yong, C., Hyoungseok, J., Sejin, J., Eum Ji, K., Jeongseop, K., and Yun Ha, J. Byungsoo, K., Murim, C., Ja Wook, K. (2023). Distinct prefrontal projections conversely orchestrate social competition and hierarchy. BioRxiv [Preprint]. doi: https://doi.org/10.1101/2023.01.04.522808

184. Narayanan, N. S., Guarnieri, D. J., & DiLeone, R. J. (2010). Metabolic hormones, dopamine circuits, and feeding. *Frontiers in Neuroendocrinology, 31*(1), 104–112. doi:10.1016/j.yfrne.2009.10.004

185. Stern, E. R., Welsh, R. C., Gonzalez, R., Fitzgerald, K. D., Abelson, J. L., and Taylor, S. F. (2013). Subjective uncertainty and limbic hyperactivation in obsessive-compulsive disorder. *Hum. Brain. Mapp. 34*, 1956–1970. doi: 10.1002/hbm.22038

186. Apergis-Schoute, A. M., Bijleveld, B., Gillan, C. M., Fineberg, N. A., Sahakian, B. J., and Robbins, T. W. (2018). Hyperconnectivity of the ventromedial prefrontal cortex in obsessive-compulsive disorder. *Brain Neurosci. Adv. 2*, 1–10. doi:10.1177/2398212818808710

187. Zamorano, F., Billeke, P., Kausel, L., Larrain, J., Stecher, X., Jose, M. H., López, V., Carrasco, X., Aboitiz, F. (2017). Lateral prefrontal activity as a compensatory strategy for deficits of cortical processing in attention deficit hyperactivity disorder. *Science. 7*, 7181. doi: 10.1038/s41598-017-07681-z