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Type 2 Diabetes Mellitus Is Associated with Social Recognition Memory Deficit and Altered Dopaminergic Neurotransmission in the Amygdala

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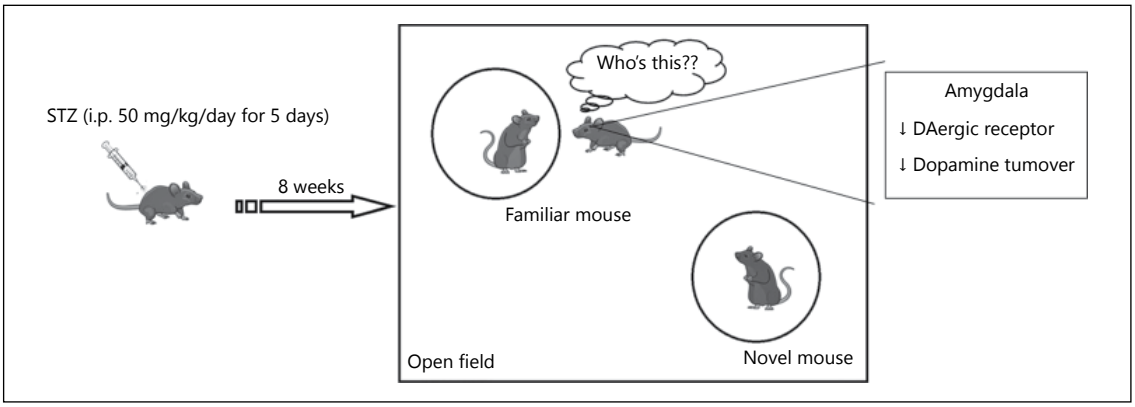
Keywords
Diabetes mellitus · Social recognition memory · Dopaminergic receptors · Dopamine turnover · Amygdala

Abstract
Objective: Diabetic neuropathy is a chronic and often disabling condition that affects a significant number of individuals with diabetes mellitus (DM). It is now established that DM causes various CNS complications like Alzheimer's, dementia, anxiety, depression, neurodegeneration, mood disorders, cognitive dysfunctioning, and so on. Since amygdala and dopaminergic circuitry are critical in controlling several aspects of social behavior, even social recognition memory (SRM), we aimed to study the expression analysis of dopaminergic circuitry in amygdala using real-time polymerase chain reaction. ***Material and Methods:*** Animals were divided into 2 age- and weight-matched groups: group I-control group and group II-diabetic group. Diabetes was induced by injecting 50 mg/kg streptozotocin (STZ; in 0.1 mL ice cold citrate buffer, pH 4.5) i.p. for 5 consecutive days. Behavioral tests were performed 8 weeks after diabetes was introduced. On day 60, animals were sacrificed, amygdala was dissected, and the total RNA was isolated. Expression analysis was car-



Graphical abstract: diabetic complications affecting recognition memory.

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ried out using real time PCR. ***Results:*** No significant changes were observed in social interaction and social isolation aspects of diabetic mice, but SRM was significantly dysregulated. Additionally, we found that dopaminergic neurotransmission (dopaminergic receptor expression and expression of enzymes controlling dopamine turnover) was significantly downregulated in the amygdala of STZ mice as compared to controls. ***Conclusion:*** We hypothesize that the altered SRM could be due to the dysregulated dopaminergic circuitry in amygdala, although a detailed investigation is required to establish a causal relationship.

Introduction

Considered for decades a disease of the peripheral nervous system, there is now mounting evidence that type 2 diabetes mellitus (T2DM) has a CNS pathology too [1]. T2DM is now known to be associated with several neurological complications like Alzheimer's, impaired learning and memory, cognitive dysfunctions, delayed executive functions, impeded information-processing speed, neurodegeneration, and neurochemical and neuro-structural abnormalities [2–9]. The underlying pathophysiology of CNS complications in T2DM is not completely elucidated, but plausibly it is diseases like hyperglycemia, vasculature disease, hypoglycemia, and insulin resistance that play critical roles [10].

Although much insight is available on the cognitive dysfunction in patients with T2DM, more needs to be understood about how it affects the social aspect of human behavior. In this context, social approach (sociability and preference for social novelty) and social recognition memory (SRM) are fundamental aspects of mammalian social behavior. Sociability is the inherent property of a rodent to form bonding with a conspecific, and preference for social novelty refers to a rodent's inclination toward a novel conspecific rather than a familiar one, just like in humans, and can be evaluated using Crawley's 3-chambered social approach task [11].

SRM is the organism's ability to recognize familiar conspecifics and is critical for many forms of social interaction including reproduction, the establishment of hierarchy dominance, and pair bonding, marking of territory, and so on. The social recognition ability of rodents can be observed in the laboratory by measuring the reduction in the amount of time spent investigating a familiar conspecific as compared to a novel conspecific [12]. Social behavior and especially SRM is mediated in most mammals by the olfactory system, which has strong innervations in the amygdala [13]. The amygdala is a principal component of social cognitive circuitry in both animal and humans and is involved in regulating emotional and social behaviors [14]. The amygdala, as a seat of emotion processing and information relay, is a likely candidate for dysfunction in T2DM-associated CNS complications. Increasing evidence indicates the role of the amygdala in the CNS pathology of diabetes [15–17]. Additionally, dopamine (DA) is of high importance because it extensively innervates the brain, the sequel to which is a wide array of behavioral parameters like cognition, emotion, perception, motivation, reward, sleep, and so on (reviewed in [18]). Further, the amygdala is not only involved in processing emotional stimuli but also involved in interacting with the dopaminergic system to assign salience to a particular stimulus [19, 20]. The amygdala is extensively connected to dopaminergic nuclei, including the nucleus accumbens and ventral tegmental area [20–22]. Additional support for creating an effect of the dopaminergic system on amygdala function comes from reports that the dopaminergic state in patients with Parkinson's disease affects amygdala responsiveness during emotion perception tasks [23]. In CNS disorders like schizophrenia, structural and functional studies demonstrate abnormalities in the amygdala and dopaminergic signaling (reviewed in [24]). Therefore, we intended to understand and evaluate the effect of T2DM-associated neurological complications on dopaminergic circuitry via dopaminergic receptor expression and dopamine turnover (dopaminergic neurotransmission) in the amygdala. Dopaminergic turnover was analyzed using expression levels of genes responsible for dopamine synthesis and metabolism, that is, aromatic L-amino acid decarboxylase (AADC) was targeted as dopamine synthesis-associated gene, while catechol-O-methyl transferase (COMT), monoamine oxidase A and B (MAO-A and MAO-B) were targeted for metabolism. Genes targeted for studying dopaminergic circuitry and their functions are mentioned in Table 1. We hypothesize that dopaminergic pathways in amygdala play a critical role in social behavior and any abnormalities in this network result in impaired emotional salience processing with consequent SRM deficits. In this study, we propose a model of social behavioral dysfunction in diabetes and discuss its therapeutic implications.

Materials and Methods

Animals

Adult male Swiss albino mice, 6–8 weeks of age, weighing 25–35 g were housed in the animal house of Jaypee University of Informa-

Table 1. Targeted genes of the dopamine circuitry and their functions

Name	Function
D1 like receptors (D1 and D5 receptor)	Locomotion, reward reinforcement, learning and memory, rennin secretion
D2 like receptors (D2, D3, and D4 receptor)	Locomotion, reward reinforcement, learning and memory, cognition and emotion
AADC	Converts L-DOPA to dopamine
COMT	Metabolizes dopamine to 3-MT Metabolizes DOPAC to HVA
MAO-A and MAO-B	Metabolizes dopamine to DOPAC Metabolizes 3-MT to HVA
DARP	Mediator in dopaminergic signaling cascade Necessary for development, recovery, and function of the brain catecholaminergic system
DAT	Pumps the neurotransmitter dopamine out of the synapse back into cytosol
Vmat2	Vmat2 is a transport protein that transports dopamine into the storage vesicles from cytosol for future neurotransmission
Pitx3	The transcription factor Pitx3 is important for the differentiation and maintenance of dopaminergic neurons during development Necessary for long-term survival and maintenance of the dopaminergic neurons Pitx3 regulates tyrosine hydroxylase expression and maintains the dopaminergic phenotype Upregulates the neurotrophic factors BDNF and GDNF, which promote dopaminergic neuron survival and confer protection against cellular insults
NURR1	NURR1, a transcription factor is critical in the development and maintenance of the dopaminergic system Regulates the expression of TH, DAT, Vmat2, and AADC, all of which are important in the synthesis and storage of dopamine

AADC, aromatic L-amino acid decarboxylase; COMT, catechol-O-methyl transferase; MAO-A and MAO-B, monoamine oxidase A and B; DARP, dopamine-releasing protein; DAT, dopamine transporter; Vmat2, vesicular monoamine transporter 2; Pitx3, paired-like homeodomain transcription factor 3; NURR1, nuclear receptor related 1 protein; 3-MT, 3-methoxytyramine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; TH, tyrosine hydroxylase; BDNF, Brain-derived neurotrophic factor; GDNF, Glial cell-derived neurotrophic factor.

tion Technology, Waknaghat, under a 12-h light/dark cycle (the lights on from 7 a.m. to 7 p.m.) at 23 ± 2°C and 60 ± 5% humidity conditions. The animals were kept in polypropylene cages and fed a standard commercial pellet diet and water ad libitum. The experimental protocol (IAEC/JUIT/2015/UB5) was approved by the Institutional Animal Ethics Committee, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh, India.

Induction of T2DM

Streptozotocin (STZ; Sigma Chemicals Co., St. Louis, MO, USA) was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 min. STZ injections were given intraperitoneally at a dose of 50 mg/kg/day for 5 consecutive days. This multiple low-dose injection of STZ is known to induce type 2 phenotype of diabetes rather than type 1 and hence can be used to study long-term complications of diabetes mellitus (DM) [25, 26]. All animals were made to fast for 20 h before STZ injection. Blood glucose levels were measured in blood samples collected from the tail vein using the Accu-Check blood glucose monitoring system (Roche Diagnostics GmbH, Germany). Animals having fasting blood glucose >200 mg/dL were selected for the study and remaining animals were excluded. Control animals received 0.1 mL ice-cold citrate buffer intraperitoneally for 5 consecutive days. Animals were regularly monitored for 8 weeks after which behavioral studies were performed.

Experimental Groups and Conditions

Animals were divided into 2 age- and weight-matched groups: group I-control group and group II-diabetic group, having 8 animals in each group. Ten days prior to the start of experimentation, animals were habituated to the experimental room on a daily basis for them to get acclimatized to the light and odor conditions of the room. Habituation period of 10 min was provided to each animal in the open field (for SRM) and 3-chambered apparatus (for social approach) having 2 steel-wire-mesh corrals were placed at equal distances from each other and walls. Animals were allowed to free-

ly explore the field and corrals. Between each habituation, the entire field and corrals were cleaned with 70% ethanol to avoid any olfactory cues. Entire habituation and experimentations were performed between 7 a.m. and 1 p.m.

Social Approach

Social approach was tested in a 3-chambered apparatus using methods previously described with a slight modification [11]. The apparatus (60 × 23 × 23 cm [h]) was a rectangular, 3-chambered box made from matte white finish acrylic. Opaque retractable doors (12 × 33 cm) were designed to create optimum entryways and encourage exploration across chamber openings (5 × 10 cm). The test was performed using same-sex animals. Subjects in the home cage were placed in the testing room 1 h before behavioral analysis for habituation and to minimize effects of stress. Testing procedures comprised the 3 following phases:

a. Habituation: doors to side chambers were closed and the subject mouse was placed in the center chamber and allowed to freely move between the chambers. After 10 min of habituation, the test animal was guided back to the center chamber, and doors were closed. Urine was soaked up, fecal pellets were removed, and side chambers were cleaned with 20% ethanol.

b. Sociability: conspecific #1 was placed under a steel-wire-mesh corral in the left chamber and the novel object was placed in the right chamber. The subject was allowed to freely investigate the chambers for 10 min. After this, the subject mouse was guided back to the center chamber, and doors were closed. Corral and novel objects were removed for cleaning and conspecific #1 was returned to the home cage. Urine was soaked up, fecal pellets were removed, and side chambers were cleaned with 20% ethanol. The time spent by a subject mouse with the conspecific or novel object was calculated.

c. Social novelty: new corrals were added, one to each side chamber. Conspecific #1 (now familiar to subject mouse) was placed under corral in the right chamber and conspecific #2 (novel mouse) was placed under corral in the left chamber. Plastic cups were placed on top of steel cups to prevent the subject from climbing on top. Subject mouse was allowed to freely investigate the chambers for 10 min. After this, the subject was guided back to the center chamber and doors were closed. All animals were returned to home cages, cups were removed for cleaning, and chambers were cleaned with 20% ethanol. The time spent by a subject mouse with familiar or novel conspecific was calculated.

SRM Test

SRM was performed on the basis of a previously reported method [27] with some modifications. The test mouse was placed in the open field (50 × 50 × 25 cm [h]) having 2 corrals and was allowed to explore freely for 5 min. One of the corrals was then removed and replaced in the same place with stimulus conspecific inside it. The test mouse was again allowed to explore the entire field for 10 min to familiarize itself with the stimulus conspecific, after which both the test and stimulus mice were removed from the field. Recognition memory was assessed 24 h after the initial interaction of the test and stimulus mice. For this, the test mouse was again placed in the open field and was allowed to explore the arena for 5 min. Both the corrals were now placed in the same position with one conspecific in each corral. One corral housed the same stimulus conspecific (now familiar to test mouse) and another housed a novel conspecific. The test animal was allowed to explore for 10 min and an entire test session was recorded with an overhead video camera. SRM is the ability of an animal to remember a previously encountered conspecific and thus, SRM was evaluated in terms of percent preference to novel conspecific over familiar conspecific by using the following equation:

% Novelty preference = Time spent exploring novel conspecific × 100

(Time spent exploring novel conspecific + familiar conspecific)

Real-Time Quantitative Reverse Transcription PCR

After behavioral studies were conducted, the animals were sacrificed, the amygdala was dissected, and the total RNA was isolated using TRI reagent (Invitrogen). The integrity of RNA was checked on 2% agarose gel and quantified using NanoDrop (ND-2000C Spectrophotometer, Thermo Scientific). The reverse transcription of 3 μg of total RNA was performed using the First strand cDNA synthesis kit (Fermentas life-sciences). qPCR amplifications were performed in a CFX96TM real-time PCR Detection System (Bio-Rad) using the iQTM SYBR green supermix (Bio-Rad) and a set of specific primers (Table 2). Reactions were carried out in total volumes of 12.5 μL and 2.5 pM of each primer and 1 μL of diluted cDNA template containing 100 ng cDNA were included. The thermal cycler conditions for all the genes used in this study are mentioned in Table 3. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the internal standard.

Statistical Analyses

All statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, Inc., USA). Data are presented as mean ± SD of $n = 5$ for gene expression analysis and $n = 8$ for behavioral analysis. Statistical significance was determined using one-way ANOVA, followed by the post hoc Dunnett's test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and nonsignificant [ns], $p > 0.05$).

Results

Social Approach

a. Sociability (Fig. 1a): we observed that sociability profile of STZ mice was normal as compared to that of control mice. STZ mice spent significantly ($p < 0.001$) more time with conspecific as compared to the novel object. There were no significant differences in time spent with conspecific between STZ and control mice. This indicates that the natural tendency of mice in terms of who they prefer is not altered by the central complications of DM.

b. Preference for social novelty (Fig. 1b): Similar to sociability profile, preference for social novelty was also observed to be nonsignificantly different between STZ and control mice. Both groups of mice spent more time investigating a novel conspecific ($p < 0.01$) as compared to a familiar one. These results indicate that central diabetic neuropathy has no effect on social approach.

Table 2. List and sequence of primers of genes involved in dopaminergic neurotransmission

Name	Primer sequence	Primer bank ID	Size, bp
D1	FP 5′-3′ TGGCACAAGGCAAAACCTACA RP 5′-3′ CTGCTCAACCTCGTGTCACA	164607143c2	92
D2	FP 5′-3′ CAAGCGCCGAGTTACTGTCAT RP 5′-3′ ATGGAGGAGTAGACCACGAAG	148747211c3	141
D3	FP 5′-3′ TGGGGCAGAAAACTCCACTG RP 5′-3′ TACCAGACCGTTGCCAAAGAT	6681220c1	106
D4	FP 5′-3′ GCCTGGAGAACCGAGACTATG RP 5′-3′ CGGCTGTGAAGTTTGGTGTG	6681223a1	142
D5	FP 5′-3′ CTCGGCAACGTCCTAGTGTG RP 5′-3′ GAGACGGCCAGAGATACGATG	142371845c1	92
COMT	FP 5′-3′ CTGGGGCTTGGTGGCTATTG RP 5′-3′ CTTTTGCGTCACCCACGTTC	161484633c1	200
AADC	FP 5′-3′ AGCTGACTATCTGGATGGCAT RP 5′-3′ ACCCCTGGCATGATTATCTTCT	299522783c1	153
MAO-A	FP 5′-3′ TGGAGCTTATGTGGGACCAAC RP 5′-3′ AGACGCTCATTGACATTCACTTT	255759901c2	93
MAO-B	FP 5′-3′ GGGCCAACCCAGAATCGTATC RP 5′-3′ GTATCAGCCGCTCAACTTCATT	257196227c2	85
DAT	FP 5′-3′ GATGCACATAGCAGCAACTCT RP 5′-3′ GCACACCACGCTCAAAATACTC	118129796c3	91
Vmat2	FP 5′-3′ AGGGGACACCTCTTACGACC RP 5′-3′ CTGCCACTTTCGGGAACACA	270483858c2	146
DARP	FP 5′-3′ ACCCCTGCCATGCTTTTCC RP 5′-3′ TTGGGTCTCTTCGACTTTGGG	21536255c1	110
Pitx3	FP 5′-3′ CCTACGAGGAGGTGTACCCG RP 5′-3′ ACCGAGTTGAAGGCGAACG	158508445c2	103
NURR1	FP 5′-3′ GTGTTCAGGCGCAGTATGG RP 5′-3′ TGTATTCTCCCGAAGAGTGGTAA	213417693c1	84

COMT, catechol-O-methyl transferase; AADC, aromatic L-amino acid decarboxylase; MAO-A and MAO-B, monoamine oxidase A and B; DAT, dopamine transporter; Vmat2, vesicular monoamine transporter 2; DARP, dopamine-releasing protein; Pitx3, paired-like homeodomain transcription factor 3; NURR1, nuclear receptor related 1 protein.

SRM (Fig. 1c)

STZ mice spent relatively more time with familiar conspecific and lesser time with novel conspecific, corresponding to a significant ($p < 0.001$) reduction in percentage novelty preference as compared to control mice, indicating that the diabetic complications led to a decline in recognition memory. Based on the social approach results, the natural tendency of rodents to explore novelty rather than familiarity was retained in STZ mice, but when tested after an interval of 24 h, they seemed to have forgotten the initial interaction, which is why STZ mice were unable to differentiate between the familiar and novel conspecifics and hence unable to prioritize their interactions.

Dopaminergic Receptor Expression Analysis (Fig. 2a)

D1 and D2 receptor expressions were almost similar in control mice compared to the expressions of glyceraldehyde-3-phosphate dehydrogenase. This expression was significantly ($p < 0.001$) reduced for both D1 and D2 re-

Table 3. Thermal cycler conditions for receptor expression

Gene	Step 1	Step 2 (35 cycles)
GAPDH	95°C for 3:00 min	95°C for 10 s, 57.6°C for 30 s and 72°C for 2:20 s
D1	95°C for 3:00 min	95°C for 10 s, 61°C for 30 s and 72°C for 2:20 s
D2	95°C for 3:00 min	95°C for 10 s, 57°C for 30 s and 72°C for 2:20 s
D3	95°C for 3:00 min	95°C for 10 s, 61°C for 30 s and 72°C for 2:20 s
D4	95°C for 3:00 min	95°C for 10 s, 59°C for 30 s and 72°C for 2:20 s
D5	95°C for 3:00 min	95°C for 10 s, 55°C for 30 s and 72°C for 2:20 s
AADC	95°C for 3:00 min	95°C for 10 s, 59°C for 30 s and 72°C for 2:20 s
COMT	95°C for 3:00 min	95°C for 10 s, 59°C for 30 s and 72°C for 2:20 s
MAO-A	95°C for 3:00 min	95°C for 10 s, 58°C for 30 s and 72°C for 2:20 s
MAO-B	95°C for 3:00 min	95°C for 10 s, 58°C for 30 s and 72°C for 2:20 s
DAT	95°C for 3:00 min	95°C for 10 s, 59°C for 30 s and 72°C for 2:20 s
Vmat2	95°C for 3:00 min	95°C for 10 s, 58°C for 30 s and 72°C for 2:20 s
DARP	95°C for 3:00 min	95°C for 10 s, 61°C for 30 s and 72°C for 2:20 s
Pitx3	95°C for 3:00 min	95°C for 10 s, 60°C for 30 s and 72°C for 2:20 s
NURR1	95°C for 3:00 min	95°C for 10 s, 57°C for 30 s and 72°C for 2:20 s

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; COMT, catechol-O-methyl transferase; AADC, aromatic L-amino acid decarboxylase; MAO-A and MAO-B, monoamine oxidase A and B; DAT, dopamine transporter; Vmat2, vesicular monoamine transporter 2; DARP, dopamine-releasing protein; Pitx3, paired-like homeodomain transcription factor 3; NURR1, nuclear receptor related 1 protein.

ceptors in STZ mice. Expression levels of D3, D4, and D5 receptors were found to be approximately similar in the control group but were significantly reduced ($p < 0.001$) in diabetic mice. Overall, there was a significant ($p < 0.001$) reduction of all the dopaminergic receptors in the amygdala of diabetic mice as compared to controls.

Dopamine Turnover Analysis (Fig. 2b)

Significant downregulation in dopaminergic turnover was observed in diabetic animals as compared to controls. Dopamine turnover was analyzed using the expression of genes responsible for synthesis (AADC) and metabolism (COMT MAO-A and MAO-B). Overall, significant ($p < 0.001$) reduction in both synthesis and metabolism responsible genes of DA was observed in STZ mice as compared to those of the control mice. This indicated that synthesis of DA was reduced, but the metabolism was reduced too. This could possibly be a protective mechanism to accumulate the DA in order to compensate the corresponding loss in DA production and DAergic receptor expression.

Others (Fig. 2c)

There was a nonsignificant ($p > 0.05$) change in the expression of DARP. DAT showed a significantly ($p < 0.001$) increased expression, which could be an answer to accumulating DA in the synapses as shown by a reduction in the number of DA-metabolizing genes (COMT and MAO). Vesicular monoamine transporter 2 showed a significantly ($p < 0.001$) decreased expression meaning that DA reuptake done by DAT was in a significantly reduced amount stored in the storage vesicles. This would also mean that for future neurotransmission, a highly reduced amount of dopamine was available, since the dopamine synthesis was significantly reduced too as depicted by a reduction in expression of dopamine synthesis gene AADC. Paired-like homeodomain transcription factor 3 (Pitx3) showed a nonsignificant ($p > 0.05$) rise, but nuclear receptor related 1 protein (NURR1) showed a significant ($p < 0.001$) increase in expression. Both Pitx3 and NURR1 are essential for dopaminergic neuron survival and development and their increased expression could possibly be in response to the dopaminergic insult by the diabetic complications as evident by a high decline in dopamine receptor expression.

Discussion

The relationship between DM and brain is complex. Diabetes-associated CNS complications are characterized by impaired cognitive functions, neurodegeneration, Alzheimer's anxiety, depression, and so on. In this

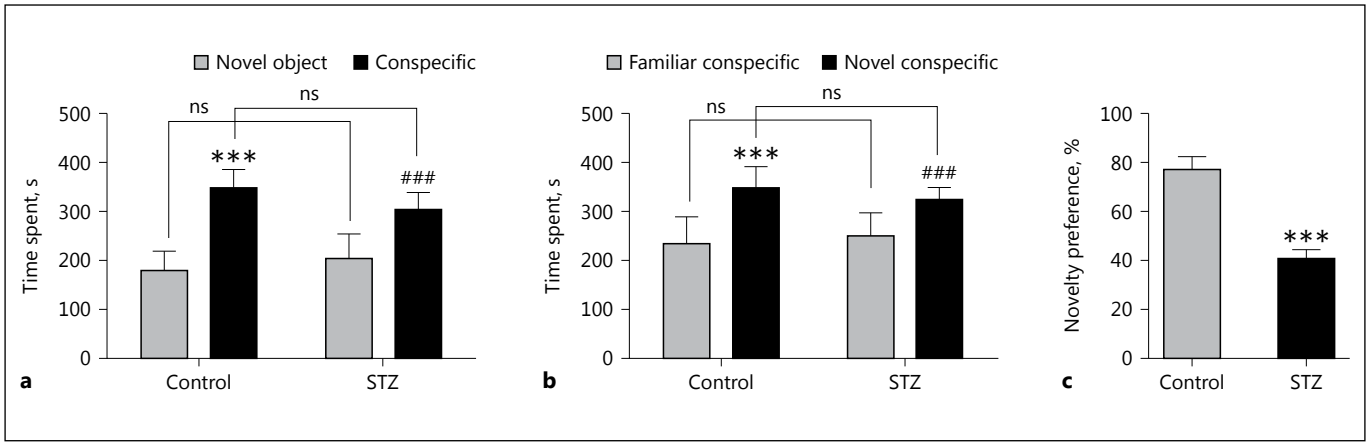

Fig. 1. Social behavior analysis of STZ and control mice. **a** Sociability, as depicted by time spent with the conspecific or novel object. **b** Preference for social novelty, as depicted by time spent with familiar or novel conspecific and (**c**) SRM as depicted by percentage novelty preference. Bars represent SD with $n = 8$. ***/### $p < 0.001$. ns, non-significant $p > 0.05$.

context, social cognitive dysfunction in patients with T2DM is increasingly recognized as a significant realm of impairment. Therefore, we aimed to study the social behavior through the evaluation of SRM in association with T2DM-associated neurological complications. Dopaminergic neurotransmission plays a pivotal role in canvassing our behavioral landscape, whereas the amygdala is the center of emotion processing and information relay and is involved in the social personality of an individual. In this study, we hypothesized that the decline in SRM in T2DM-associated CNS complications might stem through altered dopaminergic signaling in the amygdala.

Our results (Fig. 1) demonstrate that although there was a significant decline in SRM of diabetic mice, no shift in social approach was observed. On a molecular level, there was a significant disruption in dopaminergic neurotransmission, that is, dopaminergic receptor and dopamine turnover in the amygdala of diabetic mice (Fig. 2). All the dopaminergic receptors (D1–D5) showed a significantly decreased expression profile in the diabetic state. Similarly, there was a decline in both DA-synthesizing and metabolism-responsible genes, indicating that DA was produced in lesser amounts, but the metabolism was reduced too, possibly to compensate the loss of dopaminergic receptors and dopamine production. Proteins were involved in DAergic neuronal development and maintenance, functioning, and survival, and we observed an increase in diabetic condition as compared to the control group. DARP and Pitx3 showed a slight although nonsignificant increased expression. NURR1 showed a significantly increased expression. This indicates that the expression of these proteins must have increased in response to the DAergic insult as a result of long-standing diabetic complications. Our results suggest that as a result of prolonged hyperglycemia, the dopaminergic system in amygdala underwent serious damage, which resulted in an impaired emotional salience and aberrant change in social behavior as observed by SRM.

Preliminary findings suggest that while further rigorous testing has to be conducted to establish a dopamine link in T2DM, our animal model seems to indicate the existence of functional and critical circuit in amygdala where dopaminergic pathway mediates socioaffiliative behaviors. Disruption to these circuits is likely to underpin T2DM-associated behavioral disorders. It is possible that social deficits in T2DM are unlikely to be manifestations of a simple dopaminergic imbalance; targeted administration of DA agonist/antagonist may prove useful as a probe for pathophysiologic circuitry. Furthermore, given the lack of therapeutic efficacy of anti-diabetic medications for social cognitive deficits, the current research provides a rational basis for better understanding the therapeutic potential of DA in T2DM, perhaps in combination with anti-diabetics and/or developing psychosocial treatment approaches.

To our knowledge, this study demonstrated for the first time that STZ-induced diabetes produces a marked attenuation of amygdala functions mediated through dopaminergic receptors. We report our investigation of the

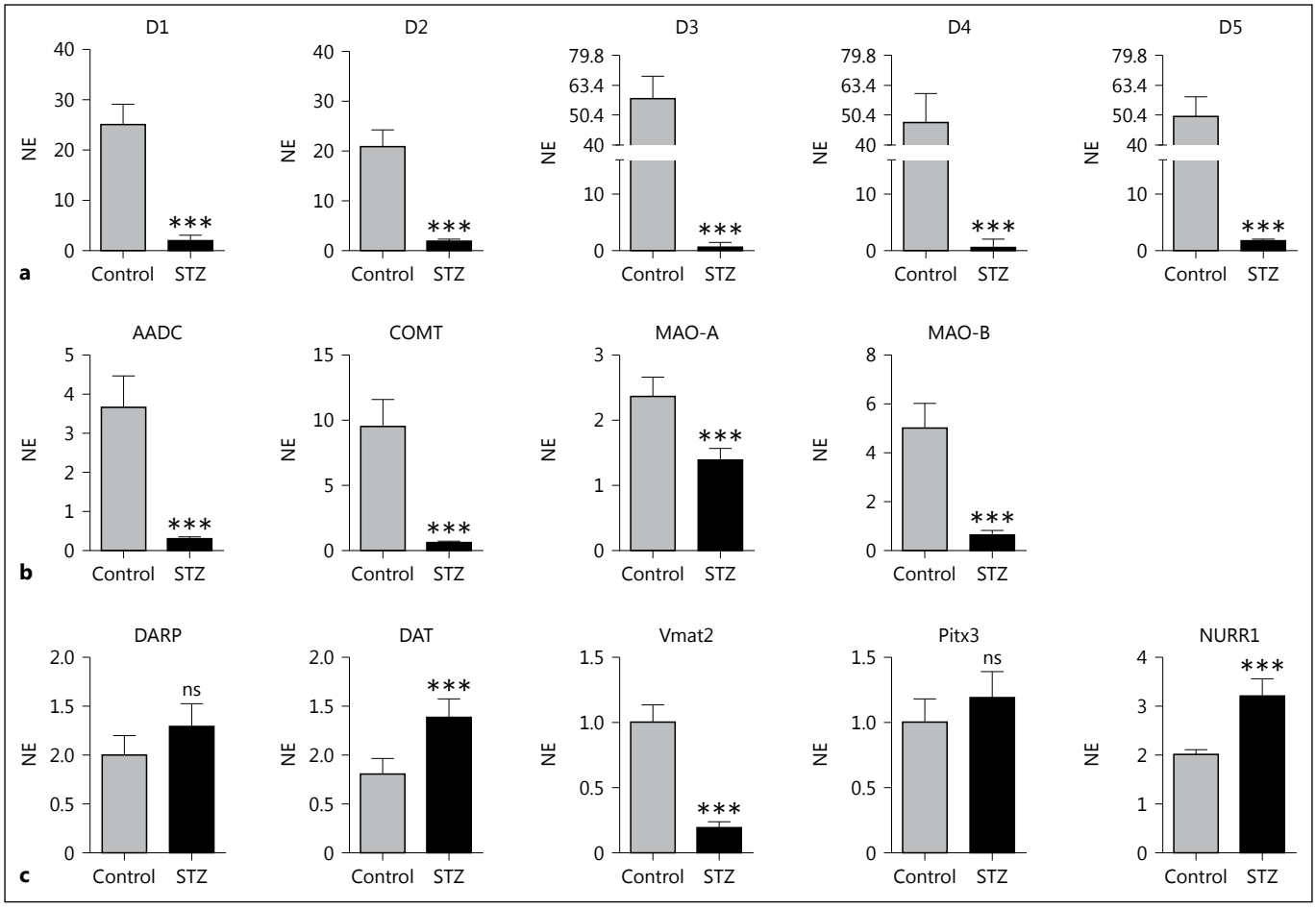

Fig. 2. Real-time PCR amplification of the mRNA from amygdala of control and STZ mice of (**a**) dopaminergic D1–D5 receptors (**b**) genes associated with dopamine turnover, namely, AADC, COMT, MAO-A, MAO-B, and (**c**) DARP, DAT, Vmat2, Pitx3, and NURR1. Values are presented as normalized expression (NE). The relative ratios of mRNA levels were calculated using the $^{\Delta\Delta}$CT method normalized with glyceraldehyde-3-phosphate dehydrogenase CT value as the internal control. Bars represent SD with $n = 5$. *** $p < 0.001$. ns, non-significant $p > 0.05$.

hypothesis that amygdala appears to be an important component of the neural system that is required for healthy social behavior. Recognition of the fact that central diabetic neuropathy is, in part, a disease that affects the whole nervous system is resulting in a critical rethinking of this disorder, thereby opening a new direction for further research.

Based on the preference for social novelty results, the natural tendency of rodents to explore novelty rather than familiarity was retained in STZ mice, but when tested after an interval of 24 h they seemed to have forgotten the initial interaction, which is why STZ mice were unable to differentiate between the familiar and novel conspecifics and hence unable to prioritize their interactions.

Conclusion

Our results suggest that central dopaminergic pathways are significantly dysregulated in diabetes, which leads to deficits in SRM, ultimately impairing neurobehavioral outcomes. This research avenue may serve as a new therapeutic target to improve socio-affiliative behaviors in patients with T2DM-associated profound social deficits.

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References

1 Selvarajah D, Wilkinson ID, Davies J, Gandhi R, Tesfaye S: Central nervous system involvement in diabetic neuropathy. Curr Diab Rep 2011;11:310–322.

2 Curb JD, Rodriguez BL, Abbott RD, Petrovitch H, Ross GW, Masaki KH, et al: Longitudinal association of vascular and Alzheimer's dementias, diabetes, and glucose tolerance. Neurology 1999;52:971–975.

3 Cukierman T, Gerstein HC, Williamson JD: Cognitive decline and dementia in diabetes – systematic overview of prospective observational studies. Diabetologia 2005;48:2460–2469.

4 Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, et al: Risk of dementia among persons with diabetes mellitus: a population-based cohort study. Am J Epidemiol 1997;145:301–308.

5 Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R: Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. Am J Epidemiol 2001;154:635–641.

6 Ott A, Stolk RP, Hofman A, van Harskamp F, Grobbee DE, Breteler MM: Association of diabetes mellitus and dementia: the Rotterdam study. Diabetologia 1996;39:1392–1397.

7 Peila R, Rodriguez BL, Launer LJ: Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia aging study. Diabetes 2002;51:1256–1262.

8 Sullivan MG (ed): Glycemic Control Shown to Prevent Dementia. 10th International Conference on Alzheimer's Disease and Related Disorders, Madrid, 2006.

9 Kodl CT, Seaquist ER: Cognitive dysfunction and diabetes mellitus. Endocr Rev 2008;29:494–511.

10 Kuusisto J, Koivisto K, Mykkanen L, Helkala EL, Vanhanen M, Hanninen T, et al: Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study. BMJ 1997;315:1045–1049.

11 Yang M, Silverman JL, Crawley JN: Automated three-chambered social approach task for mice. Curr Protoc Neurosci 2011;chapter 8:unit 8.26.

12 Gheusi G, Bluthe RM, Goodall G, Dantzer R: Social and individual recognition in rodents: methodological aspects and neurobiological bases. Behav Processes 1994;33:59–87.

13 Bielsky IF, Young LJ: Oxytocin, vasopressin, and social recognition in mammals. Peptides 2004;25:1565–1574.

14 Phelps EA, LeDoux JE: Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 2005;48:175–187.

15 Mohamadi Y, Jameie SB, Akbari M, Staji M, Moradi F, Mokhtari T, et al: Hyperglycemia decreased medial amygdala projections to medial preoptic area in experimental model of diabetes mellitus. Acta Med Iran 2015;53:1–7.

16 McNay E: Recurrent hypoglycemia increases anxiety and amygdala norepinephrine release during subsequent hypoglycemia. Front Endocrinol 2015;6:175.

17 Beeri MS, Schmeidler J, Silverman JM, Gandy S, Wysocki M, Hannigan CM, et al: Insulin in combination with other diabetes medication is associated with less Alzheimer neuropathology. Neurology 2008;71:750–757.

18 Baskerville TA, Douglas AJ: Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders. CNS Neurosci Ther 2010;16:e92–e123.

19 Ochsner KN: The social-emotional processing stream: five core constructs and their translational potential for schizophrenia and beyond. Biol Psychiatry 2008;64:48–61.

20 Laviolette SR: Dopamine modulation of emotional processing in cortical and subcortical neural circuits: evidence for a final common pathway in schizophrenia? Schizophr Bull 2007;33:971–981.

21 Reynolds GP: Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. Nature 1983;305:527–529.

22 Parkinson JA, Cardinal RN, Everitt BJ: Limbic cortical-ventral striatal systems underlying appetitive conditioning. Prog Brain Res 2000;126:263–285.

23 Tessitore A, Hariri AR, Fera F, Smith WG, Chase TN, Hyde TM, et al: Dopamine modulates the response of the human amygdala: a study in Parkinson's disease. J Neurosci 2002;22:9099–9103.

24 Rosenfeld AJ, Lieberman JA, Jarskog LF: Oxytocin, dopamine, and the amygdala: a neurofunctional model of social cognitive deficits in schizophrenia. Schizophr Bull 2011;37:1077–1087.

25 Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, et al: A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 2000;49:1390–1394.

26 Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P: Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005;52:313–320.

27 Karlsson SA, Haziri K, Hansson E, Kettunen P, Westberg L: Effects of sex and gonadectomy on social investigation and social recognition in mice. BMC Neurosci 2015;16:83.