Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: December 31, 2023

Citation: Nogueira-Souza, A., C., et al., 2023. Comparison between a *Phoneutria nigriventer* toxin treatment and galantamine treatment in a memory deficit mouse model. Medical Research Archives, [online] 11(12). https://doi.org/10.18103/mra.v11i12.4693

DOI: https://doi.org/10.18103/mra.v11i12.4693

ISSN: 2375-1924

RESEARCH ARTICLE

Comparison between a *Phoneutria nigriventer* toxin treatment and galantamine treatment in a memory deficit mouse model

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ABSTRACT

Acetylcholine modulates circuits related to cognitive functions (attention, cognitive flexibility, memory, and social interaction). Consequences of low acetylcholine levels in cholinergic synaptic clefts include memory and sociability impairments. VAChT KDHET mice exhibit diminished vesicular acetylcholine transporter protein production, which could evoke low acetylcholine quantal release and memory and sociability issues. It has been demonstrated that the PhKv toxin, isolated from the *Phoneutria nigriventer* (Brazilian wandering spider) venom, inhibits the enzyme responsible for acetylcholine hydrolysis, acetylcholinesterase. This could result in acetylcholine remaining longer in the synaptic cleft, as well as alleviating cognitive problems caused by low levels of this neurotransmitter. Hence, here we intended to investigate VAChT KDHET mice cognitive deficits in a behavioral assay - the novel object recognition task - and examine the potential effect of the PhKv toxin in memory improvement of this mouse model. First, we noted that VAChT KDHET mice showed impaired object recognition memory. We also observed that these deficits, especially those related to short-term memory in the behavioral test, are sex-related. Subsequently, to assess the effects of PhKv toxin in object recognition memory, we injected PhKv or galantamine in VAChT KDHET mice and compared their performance in the novel object recognition task. We noted that mice treated with PhKv performed similarly to mice treated with galantamine or both vehicles in this behavioral assay. Finally, we observed that mice treated with both vehicles displayed memory improvements compared to non-treated (naive) mutant mice. Together, our results imply that PhKv may have an effect on memory improvement, which might be further explored to elucidate its mechanisms of action.

Keywords: *Phoneutria nigriventer*, PhKv, spider toxin, object recognition memory, vesicular acetylcholine transporter.

1. Introduction

The vesicular acetylcholine transporter (VAChT) is an essential protein for cholinergic neurotransmission. It is a transmembrane protein that belongs to the solute carrier family 18 of the major facilitator superfamily of active transporters. It has been suggested that VAChT is a slow-type vesicular transporter. Therefore, VAChT protein expression and activity might regulate the release of acetylcholine (ACh) promptly, acting as a limiting factor in the recycling of cholinergic synaptic vesicles[1-6]. The VAChT KD (knockdown) mouse, created by Prado and collaborators (2006), is a great animal model to investigate the outcomes of decreased expression of VAChT on cholinergic neurotransmission[7]. VAChT KD mice present a reduction of the VAChT gene and protein expression, according to their genotype: VAChT KDHOM (homozygous) mice have a 65% decrease in VAChT protein expression, whereas VAChT KDHET (heterozygous) have a 45% reduction in this protein expression. The diminution of the VAChT protein production evoked an impairment in cholinergic neurotransmission, which resulted in cognitive and motor losses in VAChT KDHOM mice and only cognitive deficits in VAChT KDHET mice[7-12]. VAChT KDHET mice show features also described in Alzheimer's disease (AD) (e.g., progressive impairment of cognitive flexibility, attention span, and recognition memory[7,13,14]. Thus, these features make VAChT KDHET mice suitable for studying how cholinesterase inhibitors (e.g., donepezil, galantamine, and rivastigmine) are used as a treatment for AD patients, could benefit in reversing cognitive degeneration observed in this disorder[15].

The cholinesterase inhibitors act directly in the cholinesterases, such as acetylcholinesterase (AChE), enzymes responsible for the ACh hydrolysis. Carvajal and Inestrosa (2011) implied that AChE might be involved in the histopathology of AD. They noted that AChE interacted with Aβ (amyloid beta) peptides directly, accentuating the deposition of the insoluble peptides on plaques[16]. Aβ aggregation in the brain is also proposed as the main cause of the pathogenesis of AD ("amyloid hypothesis")[17,18]. This fundamental role of AChE implies that AChE inhibitors might act as modifying agents for this disease. Nonetheless, commercialized cholinesterase inhibitors agents exhibit plentiful side effects (e.g., nausea, diarrhea, vomiting, headache, bradycardia, syncope, and dizziness), which may be distressing for most patients. In this scenario, new cholinesterase inhibitor agents could be desirable candidates for future therapy for AD, notably those presenting fewer side effects[19-22]. PhKv, a toxin isolated from the *Phoneutria nigriventer* spider venom, has been recently investigated as a potential substitute for commercialized cholinesterase inhibitor agents[23,24].

The PhKv toxin (AECAAVYERCGKGYKRCCEE RPCKCNIVMDNCTCKKFISEL, MW = 4582.93 Da), initially named Tx3-1, is an example of neurotoxin isolated from PNV[25-27]. PhKv effects were first investigated by Kushmerick and collaborators (1999), that observed that PhKv inhibited a specific voltage-gated calcium-independent potassium channel type of current, the A-type K^+ current (I_A)[28]. Almeida and colleagues (2011) showed that PhKv could diminish the duration of cardiac arrhythmias in rats[23]. Gomes and collaborators (2013) explored the effects of PhKv on

memory. They noticed that PhKv was able to enhance memory in $A\beta_{25-35}$-treated mice without adverse effects[29]. More recently, Rigo et al. (2017) inquired about the potential analgesic effect of PhKv toxin. They noted that PhKv could reduce the capsaicin nociceptive process *ex vivo* and that inhibit AChE in mice spinal cord *in vivo*[24]. Overall, these studies show the therapeutic outcomes of PhKv toxin and its potential clinical pharmacological benefits. Therefore, here, we investigated the effects of the PhKv toxin, administered directly in the central nervous system, in improving memory shortfall in mice with cholinergic neurotransmission impairment, the VAChT KDHET mouse model.

2. Materials and methods

2.1 ANIMALS

Heterozygous VAChT mice were backcrossed with C57BL/6 animals for at least three generations. The offspring were genotyped at postnatal weeks. Animals were housed individually in clear polyethylene cages, with pine wood shaving bedding and enrichment, in a temperature-controlled room (22 ± 1°C), with 12h:12h light-dark cycles. Food and water were provided *ad libitum*. Female and male mice were used for the behavior testing aged 17 to 22 weeks. All experimental procedures were approved by the Ethics Committee on the Use of Animals at the Universidade Federal de Minas Gerais (CEUA-UFMG) under protocol number 345/2019.

2.2. NOVEL OBJECT RECOGNITION TASK

The apparatus used for assessing the ORM of the subjects was an open MDF (medium-density fiberboard) square box (40 x 40 x 40 cm) with white opaque walls and floor. Objects used were made of acrylonitrile butadiene styrene or polylactic acid, two distinct types of plastic with different shapes, textures, colors, and sizes. A lampshade placed 60 cm next to the apparatus provided constant illumination of about 15 lux, and a speaker provided background sound isolation. Animals were acclimated to the room for at least 30 min before the beginning of each trial.

The novel object recognition task (NORT) was performed according to de Jaeger and colleagues (2013), with some alterations. The task consisted of three stages: habituation (HAB), familiarization (FAM), and tests. In the HAB phase, mice were individually placed on the empty apparatus for 10 min, free to explore. After 24h of HAB, mice were introduced to two identical objects (A1 and A2), which were placed in a symmetrical position from the walls of the apparatus, for a single 10 min session. This phase was named familiarization (FAM). The last stage consisted of the tests phase, divided into a short-term memory (STM) test and a long-term memory (LTM) test. In the STM test, animals were reintroduced to the apparatus 90 min after FAM and submitted a new set of objects, a familiar object (A) and a novel object (B), placed at the exact locations during the FAM stage, for 10 min. In the LTM test, mice were reset on the apparatus 24h after FAM and exposed to two objects, a familiar object (A) and a novel object (C), for 10 min[11].

To avoid displacing the objects throughout the experiment, they were fixed with tape on the bottom, 12 cm away from the walls. Objects were used in a counterbalanced manner to prevent preference by the subjects. The apparatus and objects were narrowly

cleaned with 70% ethanol and ventilated between animals and across sessions. After each stage, animals were returned to their home cages. The time spent exploring the apparatus and the objects was recorded. Total distance traveled, time spent in the periphery, exploration time of each object, and total exploration time (sum of both times of exploration of both objects) were scored using the ANY-maze software (Stoelting Co., Illinois, IL, USA), version 7.0. The exploration time of the object was counted when mice were in direct contact with the object or when they stretched off their necks in an area 5 cm around the container, with their nose pointed towards the object. The discrimination index (DI) was used as a memory parameter. DI was calculated according to the following formula: (TN - TF)/(TN + TF), where TN is the total exploration time of the novel object, and TF is the total exploration time of the familiar object.

2.3 DRUGS AND TREATMENTS

Galantamine was purchased from Tocris Bioscience (Minneapolis, MN, USA). PhKv toxin was isolated from the *P. nigriventer* venom according to Cordeiro and colleagues (1993) by the Serviço de Proteômica e Aracnídeos, at Ezequiel Dias Foundation (Funed)[30]. Galantamine and PhKv stock solutions were prepared one day before the treatments and stored at -20°C. Galantamine (1 mg/kg) or vehicle (saline 0.9%) was administered by subcutaneous (s.c.) route (Prado et al., 2006). PhKv (100 pmol/site) or vehicle (PBS 1x) was injected via intracerebroventricular (i.c.v.) route[24,29].

The surgical procedure was performed according to Magno and collaborators (2019), with some modifications. Briefly, for the i.c.v. injection, mice were fully anesthetized with a mix of ketamine/xylazine (80 mg/kg per 8 mg/kg), through intraperitoneal (i.p.) injection. Animals were maintained on oxygen support (1 L/min) and under deep anesthesia (isoflurane 1%) during the surgical procedure. Mice were placed into a stereotaxic frame with body temperature control. To access the lateral ventricle, a craniotomy was performed by a dental drill with a 0.75 mm burr according to the following coordinates from bregma: anteroposterior (AP) -0.20 mm, mediolateral (ML) -1.00 mm, and dorsoventral (DV) -2.20 mm. PhKv or vehicle was injected through a pulled borosilicate glass micropipette. The volume injected was 3000 nL (3 uL), at an automated rate of 150 nL/min, and posterior 5 min to guarantee the diffusion of the liquid. After surgery, animals received proper post-surgical care. They were injected with a mix of ketoprofen (5 mg/kg) and Ringer's lactate solution via s.c., and were placed in a pre-warmed (37 °C) clean cage. Mice received wet food pellets placed in a small Petri dish to facilitate feeding. The experimenter closely supervised them until completely awake[31].

2.4 EXPERIMENTAL DESIGN

To assess the cognitive deficits of VAChT KDHET/WT mice, 18 animals (F = 9, M = 9) performed NORT. Mice were divided into two groups according to genotype: VAChT KDHET (n = 10) and VAChT WT (n = 8).

To evaluate the effects of the PhKv toxin on cognition, 36 VAChT KDHET mice (F = 18, M = 18) were divided into three groups: PhKv (n = 12), galantamine (n = 12), and control/sham (n = 12). The PhKv group received vehicle (saline 0.9%) s.c. and PhKv toxin (100 pmol/site) i.c.v., the galantamine group received galantamine (1 mg/kg) s.c. and

vehicle (PBS 1x) i.c.v., and the control/sham group received both vehicles s.c. and i.c.v. Before the treatments, animals performed the first and second stages of NORT (HAB and FAM, respectively). Right after FAM, mice underwent treatment with galantamine or vehicle (sterile 0.9% saline) s.c., followed by PhKv or vehicle (sterile PBS 1x) i.c.v., to prevent any effects of injection during the FAM stage (Gomes et al., 2013). After 24h of FAM, animals performed the LTM stage.

2.5 STATISTICS

Statistical analysis was performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA), version 8. Results for all measures were expressed as mean ± S.E.M. (standard error of the mean) or median with IQR (interquartile quadrant). Grubb's test (alpha = 0.05) was performed to establish significant outliers, which were excluded from the analysis, followed by the Shapiro–Wilk normality test, used to determine the normal distribution of data. Unpaired Student's t-test or Mann-Whitney test, ordinary one-way analysis of variance (ANOVA) or Kruskal-Wallis test, and ordinary two-way ANOVA were performed, depending on the experiment. Values of $p < 0.05$ were considered significant.

3. Results

3.1 VACHT KDHET MICE PRESENT SEX-RELATED IMPAIRED OBJECT RECOGNITION MEMORY

Object recognition memory (ORM) impairment is a particular feature presented by VAChT KD mice. Therefore, we submitted VAChT KDHET/WT mice to the NORT, in order to assess such cognitive impairment. This behavioral assay is divided into three stages: HAB, FAM, and test[7,8,11].

After 24h of HAB, mice were introduced to two equivalent objects (FAM stage). The total exploration time of the two objects was not statistically significant for VAChT KDHET and VAChT WT mice, demonstrating that both genotypes explored the objects likewise (**Figure 1A**). We also observed that mice had no preference for one of the objects, as expected (**Figure 1B**). 90 minutes after FAM, mice were submitted to the first test to evaluate their STM, in which they were introduced to a familiar object and a novel one. We observed that VAChT WT mice explored more both of the objects than VAChT KDHET mice (**Figure 1C**). DI was significantly distinct between genotypes, as VAChT WT mice did not show a preference for the novel object, whereas VAChT KDHET mice preferred exploring the familiar object (**Figure 1D**). After 24h of FAM, animals were submitted to the second test to evaluate their LTM, in which they investigated a familiar object and another unfamiliar object. As seen on STM, the total exploration time was significantly distinct between VAChT KDHET and VAChT WT mice (**Figure 1E**). However, mutant mice could not distinguish the objects, whereas WT mice could differentiate them (**Figure 1F**).

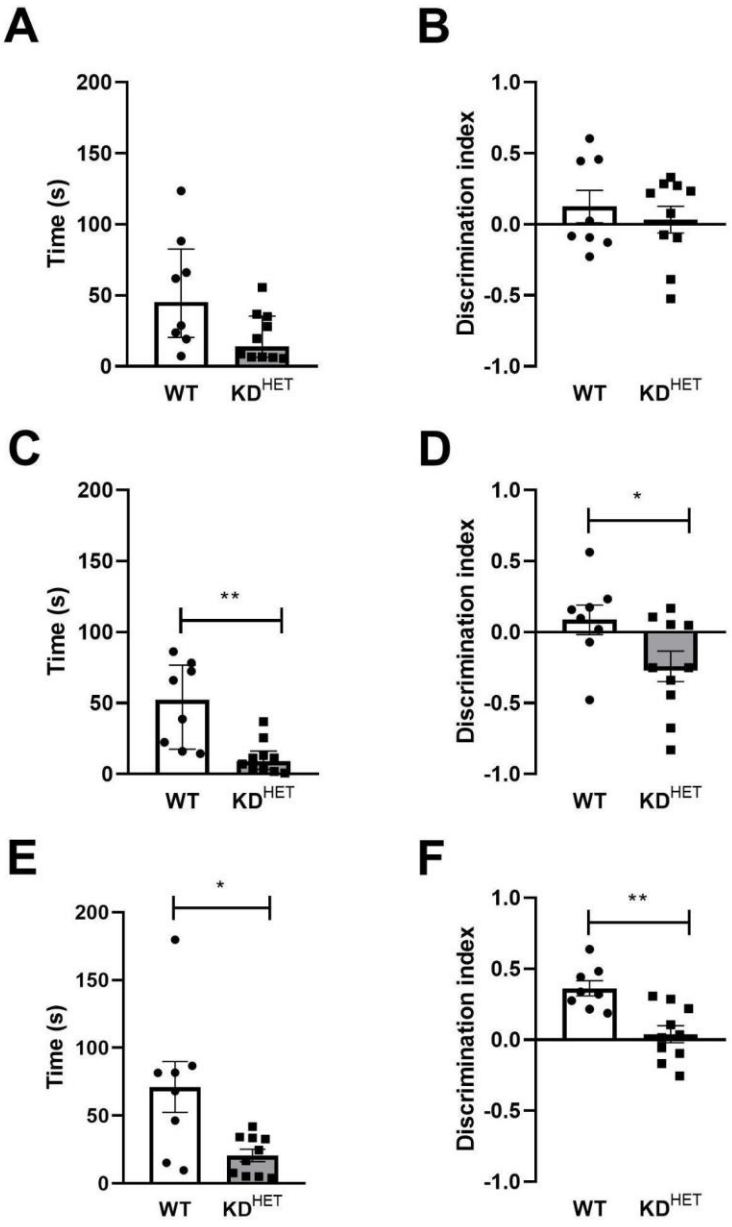

Figure 1. VAChT KD^{HET} mice exhibit diminished ORM. (A, B) Evaluation of FAM parameters: total exploration time (A) and DI (B). (C, D) STM parameters: total exploration time (C) and DI (D). (E, F) Evaluation of LTM parameters: total exploration time (E) and DI (F). (n=8-10/group)*. $p < 0.05$**, $p < 0.01$.

As Capettini and colleagues (2011) showed sexual dimorphism in VAChT KDHET mice memory, we divided the groups according to sex (Capettini et al., 2011). Comparing each sex according to the genotypes, we observed that, in the STM stage, female mutant mice displayed an ORM impairment, as they preferred to explore the familiar object rather than the novel object compared to female wild-type mice. In LTM, female VAChT KDHET mice also showed impaired recognition memory compared to female WT mice. Nonetheless, female mutant mice did not show a preference for either of the objects. In terms of male mice, we noticed that male VAChT KDHET mice exhibited memory

deficits in STM and LTM stages; however, mutant mice performance in the STM stage was not statistically significant compared to male WT mice. Nevertheless, the performance of mutant mice in the LTM stage was statistically significant. At last, comparing both sexes and genotypes, we noted that, in STM, female and male VAChT KDHET mice and male VAChT wild-type mice presented a poorer performance than female WT mice. In LTM, female and male mutant mice presented ORM deficits compared to female WT mice; furthermore, male VAChT WT mice performed similarly to female VAChT KDHET (**Figures 2A-B**).

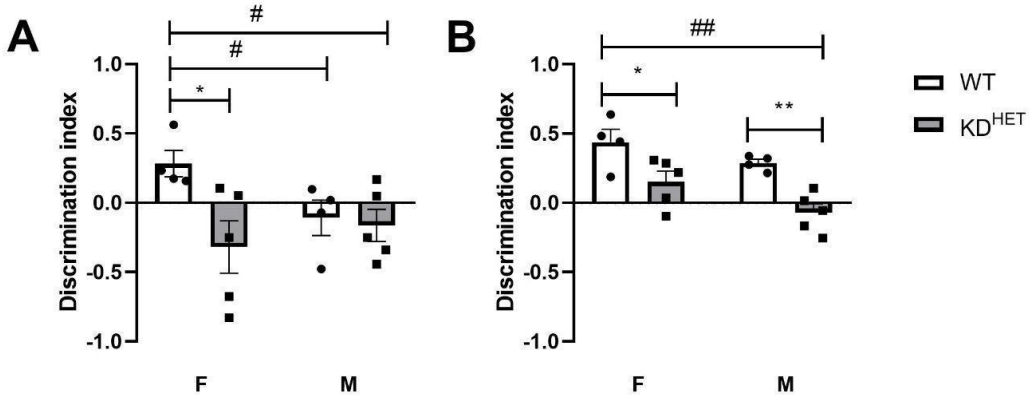

Figure 2. Male mutant mice show impaired ORM performance as do female mice. (A-B) DI according to sex in STM (A) and LTM (B). (n = 4-5/group). Statistically different performance when compared to WT mice (*), and when compared to female VAChT WT mice*. $p < 0.05$**, $p < 0.01$. # $p < 0.05$, ## $p < 0.01$.

These results imply that mutant mice seem to have STM and LTM impairment that affects their performance in NORT in both test stages.

3.2 VACHT KDHET MICE TREATED WITH PHKV VIA INTRACEREBROVENTRICULAR DID NOT SHOW A STATISTICALLY SIGNIFICANT PERFORMANCE IN NOVEL OBJECT RECOGNITION TASK COMPARED TO MICE TREATED WITH GALANTAMINE OR BOTH VEHICLES

Gomes and colleagues (2013) suggested that PhKv improved STM and LTM of Aβ25-35-treated mice[29]. Rigo and collaborators (2017) investigated the antinociceptive effect of PhKv and implied this phenomenon was associated with the inhibition of AChE[24]. As VAChT KD mice could present a cholinergic quantal release deficit, and PhKv inhibits AChE, which increases ACh levels in the synaptic cleft, we investigated the effects of PhKv on VAChT KDHET ORM.

After 24h of HAB, mice were introduced to two similar objects (FAM stage). They did not show a statistical difference in total exploration time and did not show a preference for one of the objects (**Figures 3A-B**). Right after FAM, mice were injected with galantamine (1 mg/kg, s.c.) or saline and PhKv (100 pmol/site, i.c.v.) or PBS. Mice were not submitted to the first test (STM) to allow an appropriate recovery after the surgical procedure. After 24h of FAM, mice were presented with the familiar and novel object

(LTM stage). We observed that all three groups, including control/sham, did not exhibit a statistical difference in total exploring time and showed a higher DI index in this stage than in FAM, which indicates that treated mice spent more time exploring the novel object than the familiar object **(Figures 3B-C)**.

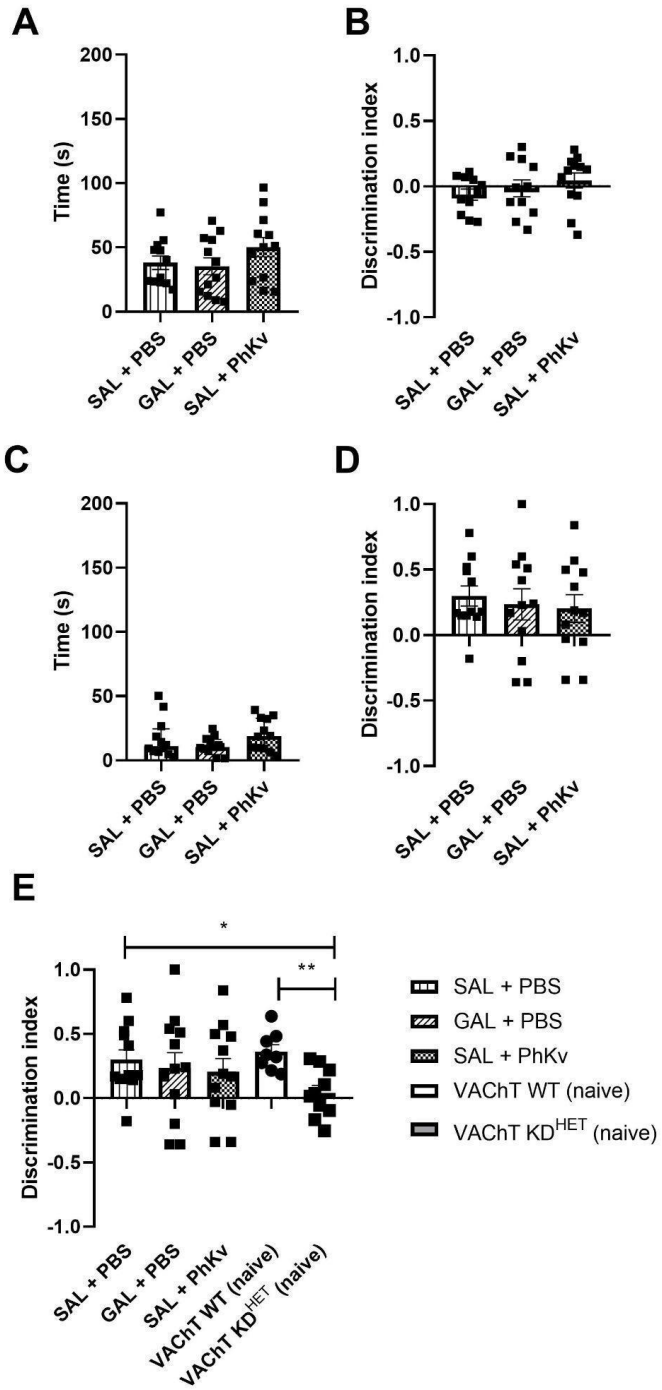

Figure 3. Mutant mice treated with PhKv performed similarly to those treated with galantamine or both vehicles. (A, B) FAM parameters: total exploration time (A) and DI (B). (C, D) LTM parameters: total exploration time (C) and DI (D). (E) DI of treated and naive VAChT KDHET mice. (n = 8-12/group)*. p <0.05**, p < 0.01.

Thus, in order to further explore these outcomes, we compared the results of this experiment to the results of the previous NORT experiment (**Figure 1F**), in which animals did not undergo surgery (naive-VAChT KDHET mice and naive-VAChT WT mice). We noted a significant difference between the control/sham group and naive-mutant mice. However, we did not observe a significant difference between galantamine or PhKv-treated mice and naive-VAChT KDHET mice.

These results imply that PhKv toxin could not wholly reverse ORM impairment in VAChT KDHET mice, as mutant mice treated with PhKv did not perform better in NORT than mutant mice that did not undergo surgery. VAChT KDHET mice treated with galantamine also did not exhibit a statistically significant performance improvement in NORT than VAChT KDHET mice that did not undergo surgery, which was unexpected.

4. Discussion

The VAChT KDHET mouse model is known for its decreased VAChT protein expression, which could directly diminish ACh release in the synaptic cleft and result in cognitive deficits. Several studies have shown that VAChT KDHET mice present impaired ORM impairment but no motor prejudice[7,9-11]. Therefore, here we evaluated the performance of VAChT KDHET mice in the NORT, in order to evaluate their cognitive deficits. NORT is primarily used to assess pharmacological and neurological modifications in the memory of rodents[32]. This assay is based on two factors of recognition memory: familiarity and recollection[33]. Access to change/novelty might elicit approach behaviors in rodents; therefore, mice are more inclined to investigate a novel object than a familiar one[34]. In NORT, mice are first presented with two familiar objects (FAM stage) and after a delay of 90 min (STM test) or 24h (LTM test), they are introduced to a novel object and a familiar one[35].

Here, we showed that mutant mice exhibited STM and LTM impairment, according to previous studies[7,9,11]. These studies did not specify the sex of mice utilized or only used male mice, a frequent practice in animal research[36]). Capettini and colleagues (2011) explored the role of sexual dimorphism on ORM in VAChT KDHET mice. They demonstrated that female VAChT KDHET mice display intact STM but LTM impairments, whereas male mutant mice present STM and LTM deficits. They also showed that female VAChT KDHET mice undergoing ovariectomy performed similarly to male mutant mice in NORT. Thus, they implied that ovarian hormones (e.g., estrogen) allowed STM maintenance by restoring the cholinergic network activity in female mice[10]. In our study, female mutant mice did not present impaired recognition memory in both stages. However, we observed that female WT mice exhibited no ORM deficits in STM and LTM stages, whereas male WT mice showed impaired performance in STM. Even though the low number of subjects in each group (n = 4-5/group) might have impacted our outcome, it has been shown that estrogen plays an essential role as a positive memory regulator, which might also explain our results[37-41]. Therefore, this outcome shows the importance of including female subjects in experiments, as they could exhibit differences in behavioral performances than seen in male subjects.

The VAChT protein is vital for cholinergic neurotransmission. It is responsible for loading ACh into synaptic vesicles. Likewise, VAChT limits ACh release, as it is a slow-type vesicular transporter. Thus, VAChT protein activity and its precedent expression may influence ACh release directly[6]. As ACh plays an essential role in cognitive functions (e.g., learning and memory), treatments that improve ACh levels in the central nervous system might result in the augmentation of the ACh release and consequently regress cognitive issues[1,42]. A primary ACh-related pharmacological target is AChE. AChE is the enzyme that breaks down ACh in choline and acetic acid, which implies the end of neurotransmission at cholinergic synapses. AChE inhibition increases ACh levels in the synaptic cleft and consequently increases the duration of cholinergic neurotransmission. AChE inhibitors are frequently utilized to treat neurodegenerative disorders, mainly AD patients since they can improve cognitive dysfunction[22,43]. Rigo and colleagues (2017) showed PhKv toxin inhibitory effect over AChE in the spinal cord of mice, which could have reduced the capsaicin nociceptive process[24]. Gomes and collaborators (2013) demonstrated that PhKv could ameliorate cognitive deficits in Aβ25-35-treated mice[29].

Therefore, we studied the potential effect of the PhKv toxin in ameliorating memory deficits in VAChT KDHET mice. In NORT, we observed that PhKv-treated mutant mice did not perform better than galantamine- (an AChE inhibitor approved for clinical use) or sham-treated animals. So, as all three groups underwent surgical processes (i.c.v. treatment), we then compared the performances of mutant mice that underwent surgery and naive mutant mice. We observed that mice treated with PhKv presented a better performance in NORT than naive-VAChT KDHET; however, the difference between both groups was not statistically significant. Unexpectedly, only the control/sham group presented a statistically significant improvement in performance when compared to VAChT KDHET mice that did not undergo surgery.

In the surgical procedure, we utilized a mix of ketamine/xylazine and isoflurane to anesthetize the animals and maintain them under deep anesthesia. Although the anesthetic protocols we used are safe for mice[44], anesthetic drugs might affect cognition. Thus, we decided to investigate the effects of each one of them on memory. Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist[45]. Yang et al. (2018) showed that ketamine (5 mg/kg, i.p.) improved memory dysfunction in a depression mouse model[46]. Contrariwise, several studies have identified adverse effects of the subanesthetic administration of ketamine on memory and learning[47].

In terms of object recognition in rodents, as NMDA receptors are directly involved in the formation of ORM, especially in the LTM stage, ketamine may affect the performance of mice in the NORT behavioral assay[48,49]. Most authors suggested that acute or chronic treatment with a subanesthetic (lower than 80 mg/kg) or anesthetic (equal or higher than 80 mg/kg) dose of ketamine, i.p., in rodents, implied ORM deficits[47,50-53]. Fan and collaborators (2021) showed that a single subanesthetic ketamine dose (10 mg/kg, i.p.) administered immediately after the second FAM stage enhanced ORM in mice[54]. Shi and

colleagues (2021) found a similar result in rats that performed the Morris water maze test, a behavioral assay utilized to assess spatial memory[55]. Ketamine might also assist ACh liberation in the hippocampus, mainly because of dopamine increase, which could help memory consolidation in rodents; however, in clinically efficient concentrations, it may as well inhibit ACh release mediated by the NMDA receptor, as demonstrated *in vitro* by Furuya and collaborators (1999)[56]. Lastly, despite presenting a weaker affinity for ACh receptors than NMDA receptor binding site, ketamine exhibits a direct inhibiting effect on both nicotinic and muscarinic receptors, which may as well impact memory[56-59]. So, the absence of consensus in the literature suggests that more pre-clinical studies should be performed in order to clarify the role of ketamine in memory. As to the other anesthetics used in our study, xylazine, and isoflurane, there was no evidence found in the literature that the acute use of each one of them, or both, could influence the performance of rodents in cognitive behavioral assays.

We also observed that VAChT KDHET mice that were treated with galantamine (1 mg/kg, s.c.) did not present an improvement in LTM stage performance, contrary to what has been suggested in previous studies[7,11]. Galantamine is an AChE reversible inhibitor and an allosteric modulator of neuronal nAChR. This dual pharmacological mechanism increases cholinergic transmission in the central nervous system and improves cognition[60]. Moriguchi and colleagues (2004) divulged that galantamine potentiates the actions of the NMDA receptor, which could also be partially responsible for the cognitive improvements seen in AD patients[61]. As previously seen, ketamine is a non-competitive NMDA receptor antagonist (i.e., it binds to the receptor blocking the NMDA receptor channel activity)[62]. Thus, both drugs could interact, reducing the effects promoted by galantamine. Nikiforuk et al. (2016) showed that a single galantamine injection (1 and 3 mg/kg, i.p.) could improve ORM in a ketamine-induced (20 mg, i.p.) schizophrenia-like rat model[63]. Nevertheless, there is no evidence in the literature about galantamine (1 mg/kg, s.c.) and ketamine (80 mg/kg, i.p.) concomitant treatment and its effects in rodents. Thus, this reasonable interaction – as well as the possible interaction between ketamine and the PhKv toxin – might be further studied.

As we showed, only a few studies showed ORM deficits in VAChT KDHET mice[7,9,11]. None of them investigated the mechanisms behind these impairments. Lima et al. (2010) suggested that quantal ACh content and size were reduced, which decreased ACh release in VAChT KD mice; however, they only utilized VAChT KDHOM mice in their studies[64]. Therefore, to understand the results achieved in our study, we suggest an additional inquiry into the molecular mechanisms underlying the deficits in VAChT KDHET mice observed herein.

5. Conclusions

We demonstrated that mutant mice presented sex-related impaired ORM. We also showed that mutant mice treated with PhKv performed similarly in the NORT compared to VAChT KDHET mice treated with galantamine or both vehicles. Furthermore, we presented that the sham

treatment was able to improve LTM, as the control/sham group displayed a significant improvement in discriminating the familiar and novel objects compared to naive VAChT KD mice; however, the PhKv treatment did not evoke a memory improvement as seen in the control/sham group animals. Therefore, we imply that the surgical procedure might have impacted our results. We also propose that the mechanisms behind VAChT KDHET mice cognitive impairments might be explored more. Lastly, in order to explicate the potential beneficial effects of PhKv toxin in mice cognition, we suggest that less invasive routes of administration would be considered in future studies (e.g., intravenous).

Conflicts of Interest Statement:

The authors declare no conflict of interest.

Acknowledgements Statement:

The authors would like to thank the Serviço de Proteômica e Aracnídeos (Funed) for providing us with the PhKv toxin, as well as the Laboratório de Biologia de Neurotransmissão (ICB-UFMG) for donating us VAChT KD mice to establish our mouse colony at the Núcleo de Experimentação Animal (FM-UFMG).

Funding Statement:

This work was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) [grant number 07/2021].

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