A Novel ^{18}F- Fallypride PET Imaging Method to Study Dopamine Release in SD Rats Induced by Alkaloids and Nicotine Salts

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Abstract

Dopamine release plays an important role in regulating neuronal behaviors behind drug addiction and abuse. Plant alkaloids and nicotine salts administrations have been reported to exert significant effects on dopamine release in human and animal brains. However, in vivo detection of dopamine in the brain is challenging and mostly invasive, which greatly limit its wide application to study drug-induced neurological mechanisms. A novel ^{18}F- Fallypride positron emission tomography (PET) imaging method was demonstrated for the detection the dopamine secretion in SD rats. The effects of four alkaloids /nicotine salts (nicotine, nicotine benzoate, caffeine and arecoline hydrobromide) on dopamine secretion in SD rats were systematically investigated based on PET imaging using 18F-Fallypride as a marker. The results showed that the effective dopamine saturation dosage of nicotine, nicotine benzoate, caffeine and arecine hydrobromide were 0.125 mg/kg, 0.150 mg/kg, 0.165 mg/kg and 0.300 mg/kg, respectively. Besides, there were also sex differences in the intensity of dopamine secretion of the four alkaloids and nicotine salts under the same dose. Additionally, animal behavior study has supported these pharmacological differences. This work provided a noninvasive real-time detection method to study dopamine excitability by neuronal stimulants in vivo to better understand addiction and abuse ability.

Introduction

Alkaloids are a group of alkaline nitrogenous compounds with significant pharmacological activities, which can form alkaloid salts in the presence of acids. They are wildly existed in the plants and food stuff, such as tobacco, poppy, lycopene, legume, alpine and palm families [1, 2]. Nicotine and its salts, caffeine, and arecoline are a kind of alkaloid, which can excite nerves and relieve fatigue, but long-term intake can cause the damage to the nervous system of the central nervous system, leading to addiction. Therefore, the dosage of alkaloid should be reasonably controlled.

Nicotine and nicotine salts intake by human can bind to nicotinic acetylcholine receptors nAChRs in the brain and promote the release of dopamine from the limbic dopamine neuron system in the midbrain, resulting in a pronounced excitatory effects [3, 4]. Chronic exposures of this nature can lead to nicotine addiction. Caffeine is a non-selective adenosine receptor antagonist, which can eliminate the negative modulatory effect of adenosine on dopamine receptors, thereby stimulating dopamine production and creating an excitatory effect [5, 6]. Arecoline on the other hand binds to muscarinic acetylcholine M receptors and also nicotinic acetylcholine receptors nAChRs, promoting the release of dopamine and producing the excitatory effects [7, 8]. Although these alkaloids/salts can directly or indirectly stimulate the production of dopamine, excessive and repeated intake of alkaloids/salts will produce adverse physiological effects and dependence behaviors, while intake of alkaloids/salts under a low dose may have beneficial effects on mental alertness and will not achieve psychological satisfaction of their users [9, 10]. Therefore, accurately establishing the relationship between alkaloids/salts intake and dopamine secretion in the brain and comparing the differences in the excitatory effects of different alkaloids/salts

will provide a reference for their potential safe consumption such as in nicotine replacement therapy products and keep away irreversible drug addiction and abuse.

The excitatory effects of alkaloids/salts are mainly related to dopamine secretion, and the strength of the excitatory effect can be indirectly reflected by detecting the occupancy of dopamine D2 receptors [11–14]. For example, Fukunaga et al. proposed that dopamine D2 receptor signaling is vital for the modulation of nicotine-induced conditioned place preference (CPP) in mice. They found the strength of both calmodulin-dependent protein kinase and extracellular signal-regulated kinase increase in wild-type mice, but not in dopamine D2 receptor knockout mice, indicating that the activation of D2R is essential for the enhancement of nicotine effect [12]. Therefore, the strength of excitatory effect can be detected by analyzing the occupation of dopamine D2 receptor. Despite many previous studied focusing on the physiologically dependent behavior of alkaloids/salts [12], the detection of dopamine in animal's brain is mostly invasive in the reported studies, which greatly limits the understanding of the mechanisms involved [15]. With the development and maturity of positron emission tomography (PET) technology, non-invasive and real-time detection of the excitatory effects of psychostimulants such as alkaloids/salts in vivo has become a possibility [16–19]. As such, Sabri et al. studied cholinergic neurotransmission in neurodegenerative disorders by PET imaging [20].

In this work, a competitive inhibitor of dopamine D2 receptor, namely ^{18}F-Fallypride, was used as an imaging agent. We systematically studied the dopamine secretion in SD rats after single gavage administration of four alkaloids/salts based on the ^{18}F-Fallypride-PET imaging technology. Open field test (OFT) of SD rats has been performed to distinguish any different behaviors between the four alkaloids/salts groups. In addition, the sex difference in the dopamine secretion behaviors has been investigated.

Materials and Methods

Materials and reagents

Nicotine (chromatographic purity), caffeine (chromatographic purity) and betaine hydrobromide (chromatographic purity) were provided by Shanghai Amperexperimental Technology Co., Ltd (China); nicotine benzoate (homemade, 50% nicotine benzoate propylene glycol liquid); isoflurane was purchased by Shenzhen Reward Life Science and Technology Co. Ltd (China). SD (Sprague Dawley) rats, SPF grade, 5 weeks age, and the weight is between 200 g and 220 g, were provided by Zhaoyan (Suzhou, New Drug Research Centre Co., Ltd. and Beijing Viton Lihua Laboratory Animal Technology Co., Ltd, China). The test animal qualification certificate numbers were 202139403 and 2021120Aazz0619000345, and the animal ethical approval numbers of the test protocols were IACUC-2021-005 and IACUC-2021-008, respectively.

High performance liquid chromatograph (1260, Agilent, USA); small animal anaesthesia machine (R5501E, Shenzhen Reward Life Science and Technology Co., Ltd., China); small animal PET-CT imaging

system (Inveon, Siemens, Germany); nebulisation drug delivery instrument for large and small mice (KW-DM-YWH, Calvin Bio-technology Co., Ltd., Nanjing, China).

Test method

Preparation of imaging agent ^{18}F-Fallypride

Firstly, A IBA Cyclone® 18/9 at 18MeV cyclotron was used for ^{18}F production. The ^{18}F ions were obtained from a nuclear accelerator and transferred to the synthesis module, and then the ^{18}F ions were adsorbed by the QMA column. Secondly, the ^{18}F ions adsorbed on QMA column were washed with 1 mL of mixture solution (15 mg K2.2.2, 1.5 mg K_2CO_3, 0.6 mL acetonitrile, and 0.4 mL of water) and the solvent was evaporated. The resulting ^{18}F ions were washed twice with acetonitrile and dried to obtain the precursor. Next, 3 mg of the precursors were added into 1 mL acetonitrile and reacted in the closed vessel at 95°C for 15 min, then the solution was cooled to room temperature, diluted with water, and passed through the C18 column for pre-purification before preparative separation. The obtained crude product was drenched down from the C18 column with 1 mL of ethanol and diluted with mobile phase, and the diluted solution was fed into the preparative column for preparative separation (HPLC flow rate: 5 mL/min, acetonitrile: water = 56:44, 0.1% triethylamine); the target product peak was collected and diluted with water, and enriched again with a C18 column; the product was eluted from the C18 column to the product vial with 1 mL of ethanol and diluted with 0.9% sodium chloride injection to obtain ^{18} F-Fallypride solution, as shown in Fig. 1.

Purity determination of ^{18}F-Fallypride

After the above synthesis, the purity of ^{18}F -Fallypride was identified by HPLC. The specific HPLC identification parameters were: mobile phase A: 0.1% triethylamine aqueous solution, B: acetonitrile. Chromatographic column: Luna C18 4.6*2505 μm; wavelength: 254 nm; elution method: 0 ~ 15 min: 40% of A phase, 60% of B phase; 15 ~ 25 min: 100% of B phase.

Determination of alkaloid/salts peak times

PET static scans were performed by injecting the developer ^{18}F-Fallypride into the tail vein of SD rats, followed by transoral gavage of a given dose of alkaloids/salts (Table 1). After the administration 55 min, the SD rats were anaesthetised and subjected to static scanning using PET imaging. The uptake value of alkaloids/salts (g) in the striatum of SD rats was obtained by adjusting the scanning time parameter (uptake value (ID%/g) = (radioactivity counts of organs/radioactivity counts of injected drugs)/tissue weight) to determine the peak time of alkaloids/salts in the brain of the SD rats, which was also the optimal scanning time for PET.

The specific scanning conditions were as follows: ^{18}F-Fallypride as the radioactive tracers, a static scanning mode was adopted, and the tail vein was scanned for 5 min after the developer was given for 30 min; reconstruction mode OSEM-3D; PET axial scanning length of 127 mm, acquisition energy

window of 350–650 keV, and time window of 3.438 ns. PET reconstruction algorithm: OSEM3D/SP-PET reconstruction algorithm: OSEM3D/SP-MAP, 2 iterations, 128*128 matrix. PET image layer thickness: 0.796 mm.

Table 1
The administration doses of alkaloids/salts in SD rats and PET scanning schedules.

Alkaloids/salts	Dose administered (mg/kg)	Scanning time (h)
nicotine	0.10	0, 0.5, 1, 1.5, 2 and 4
nicotine benzoate	0.12	
caffeine	0.13	
arecoline hydrobromide	0.12	

Dopamine secretion assay with different alkaloids/salts at different doses

After injection of 18F-Fallypride developer into the tail vein of SD rats, different doses of alkaloids/salts were given to SD rats by single oral gavage administration (Table 2), and PET static scanning time was performed to observe the difference of dopamine secretion in SD rats after single oral injecting of different doses of alkaloids/salts.

Table 2
Different alkaloids/salts does in SD rats.

Alkaloids/salts	Dose administered (mg/kg)					
	1	2	3	4	5	6
nicotine	0.015	0.030	0.063	0.125	0.250	0.500
nicotine benzoate	0.020	0.040	0.075	0.150	0.300	0.600
caffeine	0.041	0.082	0.165	0.330	0.650	1.300
arecoline hydrobromide	0.020	0.040	0.075	0.150	0.300	0.600

Dopamine secretion assay of different alkaloids/salts with same doses

Each test group (nicotine group, nicotine benzoate group, caffeine group and arecoline hydrobromide group) included three male and three female SD rats. After different alkaloids/salts with the same dose were given to SD rats by the single gavage administration, ^{18}F- Fallypride developer was injected into the tail vein of SD rats, and PET static scanning was performed to observe the difference in dopamine secretion of different categories of the same dose of alkaloids/salts.

Open field test of different alkaloids/salts with same doses

Open field test (OFT) was used to determine any behavioral differences after the exposure of different alkaloids/salts with same doses. The experiments were conducted with 24 SD rats, which were randomly allocated into four groups (nicotine, nicotine benzoate, caffeine and arecoline hydrobromide) with 6 rats in each group. Each rat received a single gavage administration of 0.10 mg/kg alkaloids/salts daily. Before the test, rats were placed into the experimental areas for 30 min of adaptation. During the test, rats were separately put in the center of an open field and allowed to move freely in the area for 10 min after 55 min of alkaloids/salts administration. The central area of the open field was defined by a 100 cm×100 cm×40cm area. A suspended camera connected to a computer was used to monitor rat behavior. Between trials, the field was cleaned with 75% alcohol to ensure that imprints from previous rats did not affect subsequent trials rat behavior. Rats tracking and test data analysis were performed by OFT systems.

Results and discussion

Purity determination of developed ^{18}F- Fallypride

The developer ^{18}F-Fallypride was synthesized and its purity was analyzed by liquid chromatography, which showed that the purity of the synthesized compound was 98.70%. When its purity reached more than 95%, it was considered adequate for the developing experiments. Next, different alkaloids/salts were given to SD rats by single gavage administration, and then the high-purity ^{18}F-Fallypride developer was injected into the tail vein of SD rats. Finally, PET static scanning was carried out to observe the effect of the alkaloids/salts type and their dosages on the dopamine secretion in rate, as shown in Fig. 2.

The effect of different alkaloids/salts on dopamine secretion After the gavage administration of the alkaloids/salts in SD rats at different time points, ^{18}F-Fallypride distribution scans within the rat striatum were obtained, and the results showed that the dopamine secretion in the SD rats striatum reached the peak after the intragastrical administration for about 1 h with the lowest striatal visualizer ID%/g values, as shown in Fig. 3. For example, the visualiser ID%/g values were as low as 0.80 ± 0.09, 0.84 ± 0.02 and 0.85 ± 0.03 after nicotine, nicotine benzoate and arecoline hydrobromide gavage for 1 h, respectively. It was observed that caffeine could competitively bind to adenosine receptors, thereby increasing dopamine activities and producing the dopamine secretion peak after gavage at 1.5 h with the lowest striatal visualizer ID%/g values (0.79 ± 0.02). There was no significant difference in the visualizer ID%/g values at 1.5 h and 1 h after the caffeine gavage administration, therefore, the final time of gavage administration was defined as 1 h.

In addition, PET imaging results showed that the intensity of the ^{18}F-Fallypride developer in the striatum of the SD rat brain changed with the time after administration at the same dose, as shown in Fig. 4. The weakest intensity of imaging of the ^{18}F-Fallypride developer appeared around 1-1.5 h, which is consistent

with the results in Fig. 3. These findings indicated that the different alkaloids/salts had a significant and differential effects on the dopamine secretion in the animal's brain. More importantly, simultaneous sequential PET scanning at multiple time points was performed in real-time to achieve nondestructive evaluation of the alkaloid/salts induced dopamine secretion process.

The effect of alkaloids/salts at different doses on dopamine secretion

After the single gavage administration of the four alkaloids/salts in SD rats, the standard uptake values of striatal visualizer 18F-Fallypride in the brain displayed a nearly U- or L- or S-shaped curve. The standard uptake values of striatal visualizer 18F-Fallypride changed gradually with the increase of the alkaloids/salts doses, but there were the lowest uptake values when the gavage administration of alkaloids/salts dose reached a certain value, as shown in Fig. 5. It was concluded that the four alkaloids/salts had a significant saturating effect on the dopamine secretion which could not rise or fall unidirectionally with the increase of the dose of the alkaloids/salts. It was found that the saturating doses of nicotine, nicotinic benzoate, caffeine and arecoline hydrobromide was 0.125 mg/kg (Fig. 5a), 0.15 mg/kg (Fig. 5b), 0.165 mg/kg (Fig. 5c), and 0.30 mg/kg (Fig. 4d), respectively. The saturating effect of dopamine secretion induced by the alkaloids/salts might be related to the relative stability of the number of dopamine receptors over time [21]. When the number of ligand alkaloids/salts continued to increase, it was impossible for more receptors to bind to them, stimulating the production of more dopamine. Therefore, the saturating effect of dopamine secretion in SD rats was observed after the gavage administration.

The presence of the dopamine secretion saturating effect induced by the alkaloids/salts suggested that a single overdose intake of the alkaloids/salts did not increase excitability, thus excessive intake of alkaloids should be avoided. Therefore, to avoid potential risk of addiction and drug abuse, appropriate levels of the alkaloid/salts contents could play an important role. It has been reported that there is a relationship between the nicotine level and the levels of some other hazardous components in consuming tobacco products, for example, during smoking a cigarette. The nicotine level intake can, to a certain extent, reflect the level of hazardous components in cigarette smoke [22]. Therefore, the discovery of the saturating effect of dopamine secretion of the four alkaloids could provide a reference for evaluating the contents of such alkaloids/salts in tobacco or other consumer products for further pharmacological investigations.

Gender differences in dopamine secretion

Based on the saturating doses of dopamine secretion after the gavage administration of the four alkaloids/salts, the intersection below the saturating doses of the four alkaloids/salts was determined to be 0.10 mg/kg. This value was set as the baseline to study any sex difference in the dopamine secretion following the alkaloids/salts exposure. Again the single gavage treatment of the four alkaloids/salts at the dose in male and female SD rats followed by the PET scan. The results of the animal brain showed

that dopamine secretion was the highest after the gavage of arecoline hydrobromide, while dopamine secretion was the lowest after the gavage of caffeine. For female SD rats, it was discovered that nicotinic benzoate induced the most dopamine secretion and arecoline hydrobromide induced the least, as shown in Fig. 6. In addition, the PET imaging results showed that the imaging intensity of the ^{18}F-Fallypride developer in SD male and female rat striatum after the administration at 0.1 mg/kg doses, as shown in Fig. 7. It was clear that there was a significant gender difference in the dopamine secretion for the four alkaloids/salts. The observed differences in the amount of dopamine secretion may be related to their physiological mechanisms, while the gender difference may be associated to the number of dopamine receptors of the two sexes. For example, Okita *et al.* showed that the striatum of female smokers possessed more accessible dopamine D2 receptors than that of male smokers, and this difference led to the hypothesis that male smokers could stimulate more dopamine secretion after nicotine acquisition, resulting in different degree of nicotine dependence between male and female smokers [23]. This seemed to agree with the animal results of the present study.

The effects of nicotine and nicotine salts on dopamine secretion in SD rats were similar despite the gender differences, which could be ascribed to the identical active ingredients. Dopamine secretion in male SD rats induced by arecoline hydrobromide, nicotine and nicotine benzoate was stronger than that of female SD rats, while the dopamine secretion in female SD rats induced by caffeine was stronger than that of male SD rats. It was obvious that dopamine secretion induced by different alkaloids/salts existed gender differences. Among the observations, caffeine was a non-selective adenosine receptor antagonist, and the intake of caffeine could indirectly increase the dopamine secretion. Meanwhile, dopamine secretion induced by arecoline hydrobromide showed the minimal sex difference among these four alkaloids/salts, which may be related to the variability and affinity of muscarinic acetylcholine M receptors in the brain of female rats. This discovery would also be useful for setting appropriate targets for potential efficacy and abuse liability thresholds by regulators for such products. This work demonstrated that that excitatory behaviors of the four alkaloids/salts in rat brain as indicated by the intensity differences of the dopamine secretion, but also the gender differences of the pharmacological effects, which provides the basis for further pre-clinical assessment of these alkaloid/salts compounds.

Open field test (OFT) of SD rats following different alkaloids/salts at same doses

To distinguish any different behaviors between the four alkaloids/salts groups, OFT was performed on individual animals receiving the same dose administration [24], and the results are shown in Fig. 8. After the four different alkaloid/salts were administered, it is found that there were distinct differences in their behaviors, such as total distance covered, speed, number of stands and number of excreta by the different groups. After the administration, there was differences between female and male SD rats. For example, the total distance covered, speed and the number of stands from the male SD rats were higher than those of the female SD rats, indicating that male SD rats acquired a higher degree of excitability than the equivalent female group. In contrast, the female SD rats showed more active than that of the male SD rats after caffeine gavage, suggesting the gender differences in drug-induced pharmacological

behaviors by these different alkaloids/salts. The above OFT results are also consistent with the results of the secretion of dopamine in the brain.

Conclusions

A non-invasive and real-time assay for the detection of dopamine secretion in SD rats after a single gavage administration of alkaloids/salts was established based on ^{18}F- Fallypride-PET imaging technology. This method showed that there was a dose-effect relationship for dopamine secretion in SD rats after the single gavage of four alkaloids/salts. In addition, there was a dopamine secretion saturation effect by the four alkaloids/salts, and the saturation effect doses of nicotine, nicotine benzoate, caffeine and arecoline hydrobromide were estimated to be 0.125 mg/kg, 0.150 mg/kg, 0.165 mg/kg and 0.300 mg/kg, respectively. More interestingly, it was also found that the dopamine secretion in SD rats at the same doses showed the intensity differences and gender differences, i.e., dopamine secretion induced by arecoline hydrobromide, nicotine and nicotine benzoate was stronger in male SD than those of female SD rats, while the dopamine secretion induced by caffeine was stronger in female SD rats than that of male SD rats. Further animal behavior study supported these pharmacological differences. This work provides the basis for noninvasive and real-time detection of dopamine secretion in SD rats and the alkaloids/salts uptake does, thus potential useful in preventing the drug addiction and abuse.

Declarations

Statement

All experimental protocols were approved by Huajing Molecular Imaging & Drug Research Institutes. The study is reported in accordance with ARRIVE guidelines and all methods were performed in accordance with the relevant guidelines and regulations. Details are as follows:

SD (Sprague Dawley) rats, SPF grade, 5 weeks age, and the weight is between 200 g and 220 g, were provided by Zhaoyan (Suzhou, New Drug Research Centre Co., Ltd. and Beijing Viton Lihua Laboratory Animal Technology Co., Ltd, China). The test animal qualification certificate numbers were 202139403 and 2021120Aazz0619000345, and the animal ethical approval numbers of the test protocols were IACUC-2021-005 and IACUC-2021-008, respectively.

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Author contributions statement

Dawei Yan: conceptualization, investigation, data analysis, writing-original draft. Hui Zhang: investigation, data analysis, writing-original draft. Xiaomin Liu: investigation and data analysis. Yihan Gao: investigation and data analysis. Xiaonan Li: investigation and data analysis. Lehua Lu: data analysis. Xiabin Chen: data analysis. Yiting Qian: conceptualization, reviewing & editing the manuscript. Saijing Zheng: conceptualization, reviewing & editing the manuscript. Yi Shen: funding acquisition, finalizing the manuscript and project administration.

Ethics declarations

All experimental protocols were approved by Huajing Molecular Imaging & Drug Research Institutes. The study is reported in accordance with ARRIVE guidelines and all methods were performed in accordance with the relevant guidelines and regulations.

Competing interests

The authors declare no competing interests.

Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Additional information

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Figures

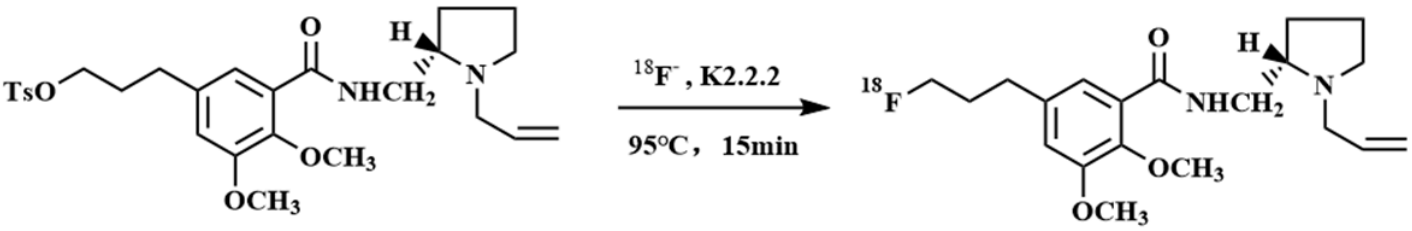

Figure 1

The synthesis route of the imaging agent ^{18}F-Fallypride

Figure 2

The Schematic diagram of experimental procedures to monitor the dopamine secretion in SD rats induced by alkaloids/salts by PET imaging.

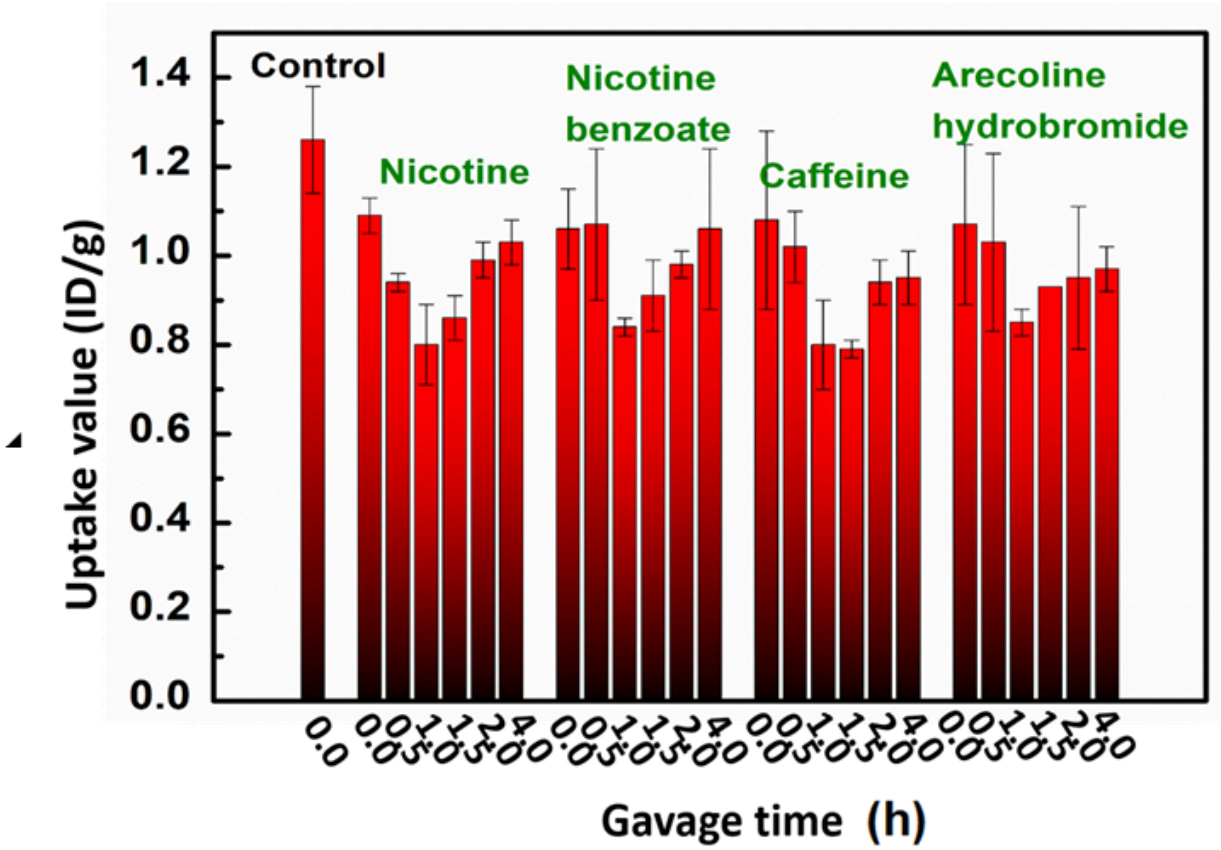

Figure 3

Distribution values of ^{18}F-Fallypride in SD rat striatum after different alkaloid/salts gavage administration at different time up to 4.0 h

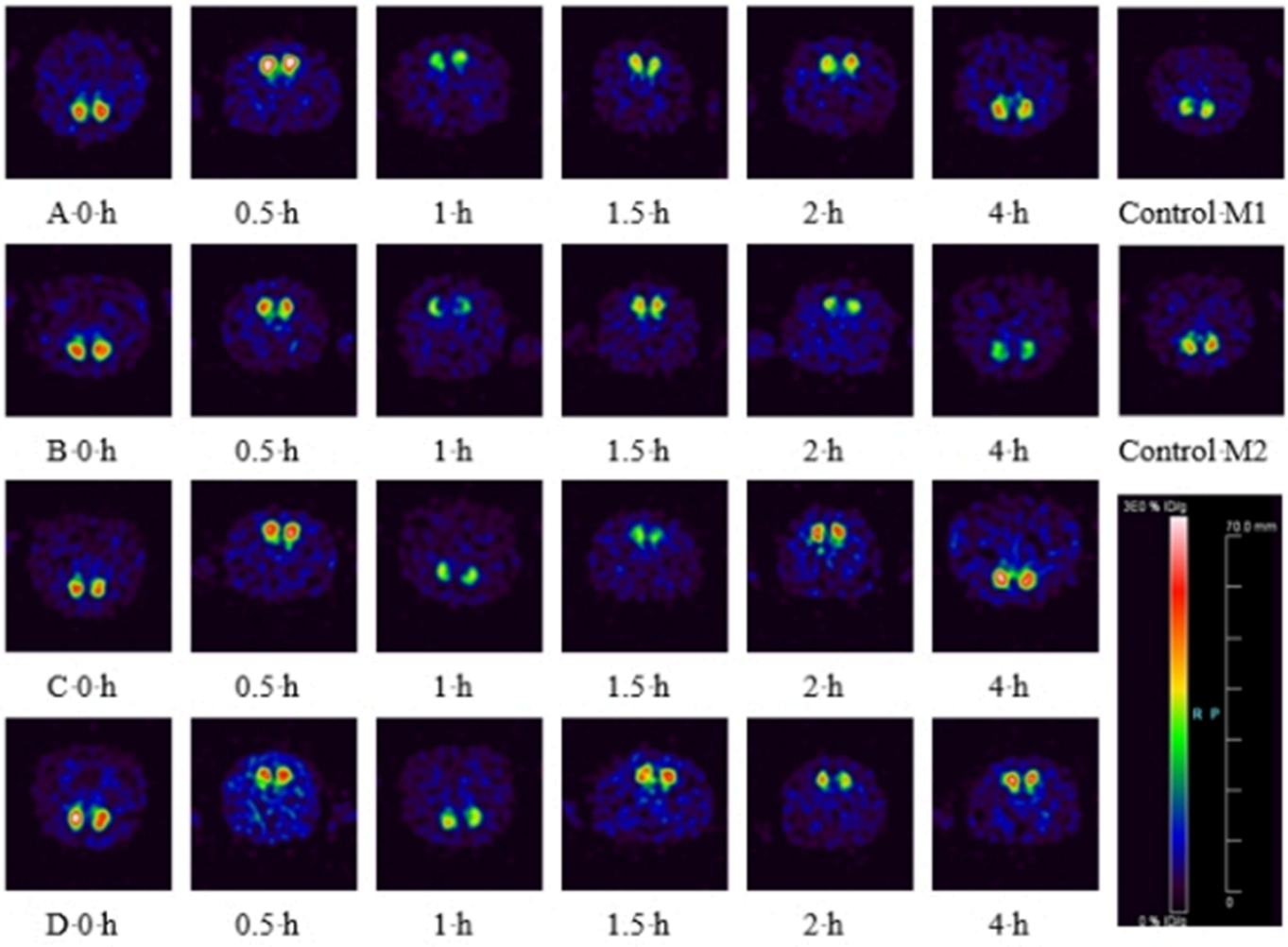

Figure 4

^{18}F-Fallypride distribution maps in rat striatum after different alkaloid/salts gavage administration at different time. (A: nicotine; B: nicotinic benzoate; C. Caffeine; D: arecoline hydrobromide)

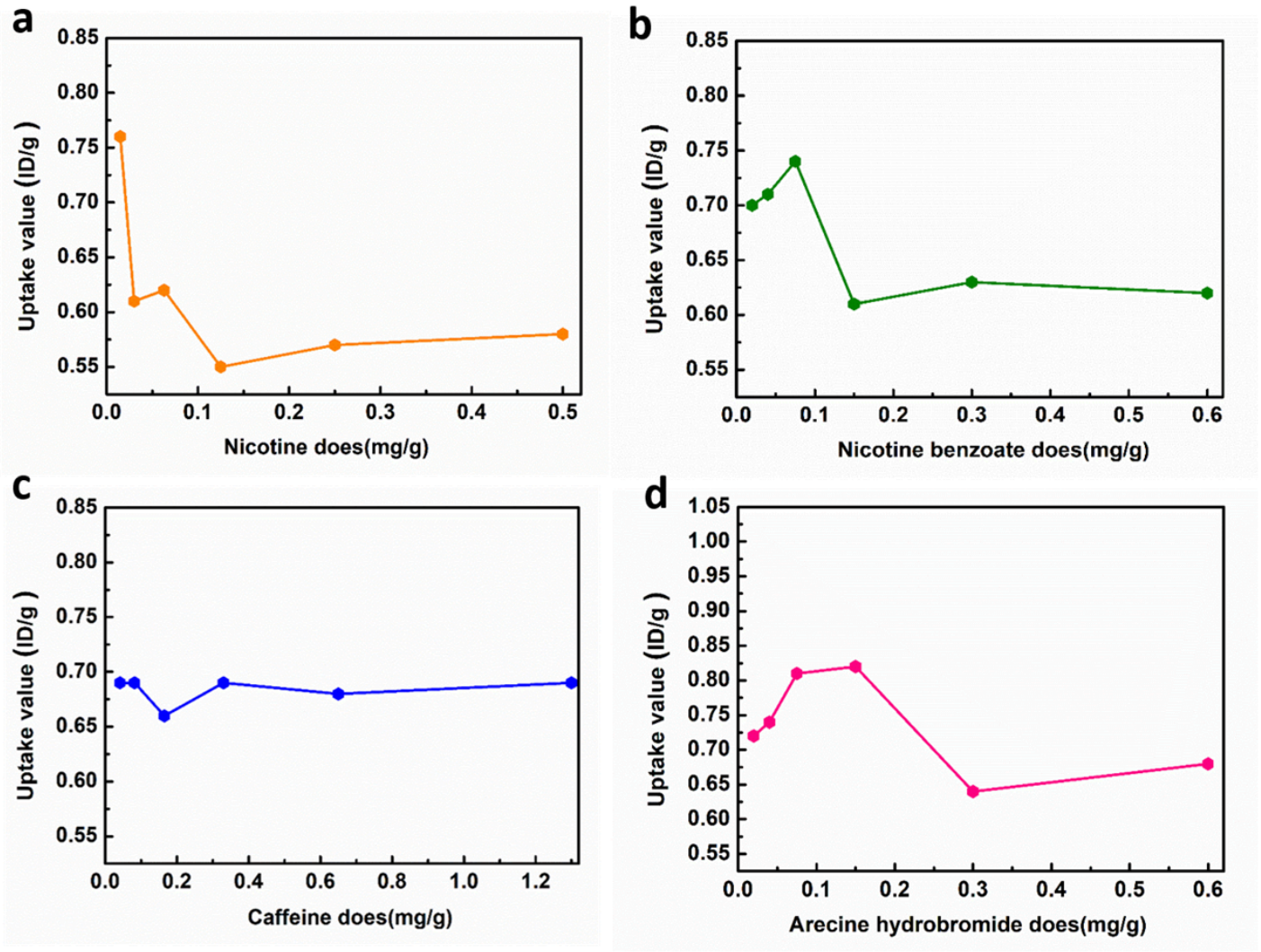

Figure 5

Distribution of ^{18}F-Fallypride in the striatum of SD rats under different alkaloids/salts doses.

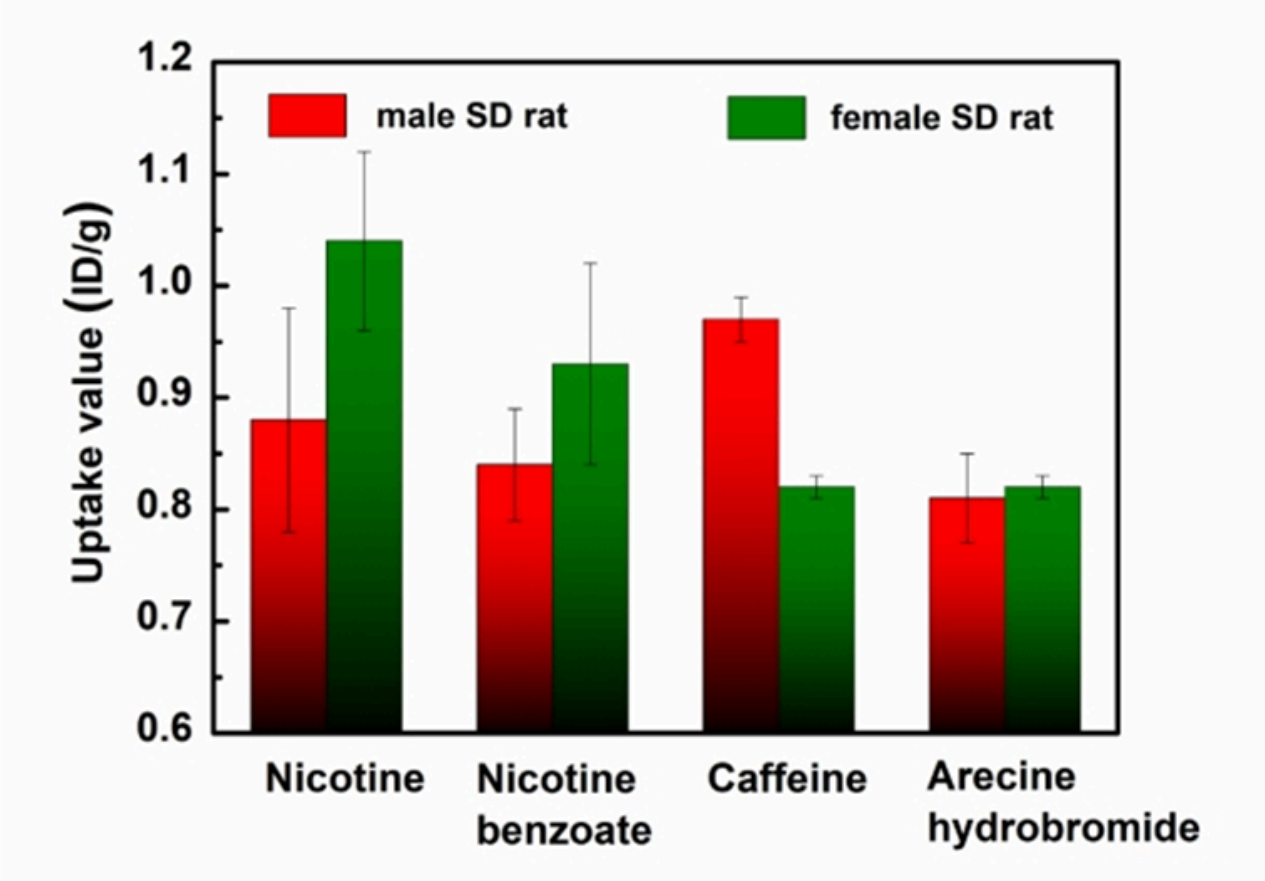

Figure 6

Distribution of ^{18}F-Fallypride in SD male and female rat striatum after gavage administration different alkaloids/salts at 0.1 mg/kg dose

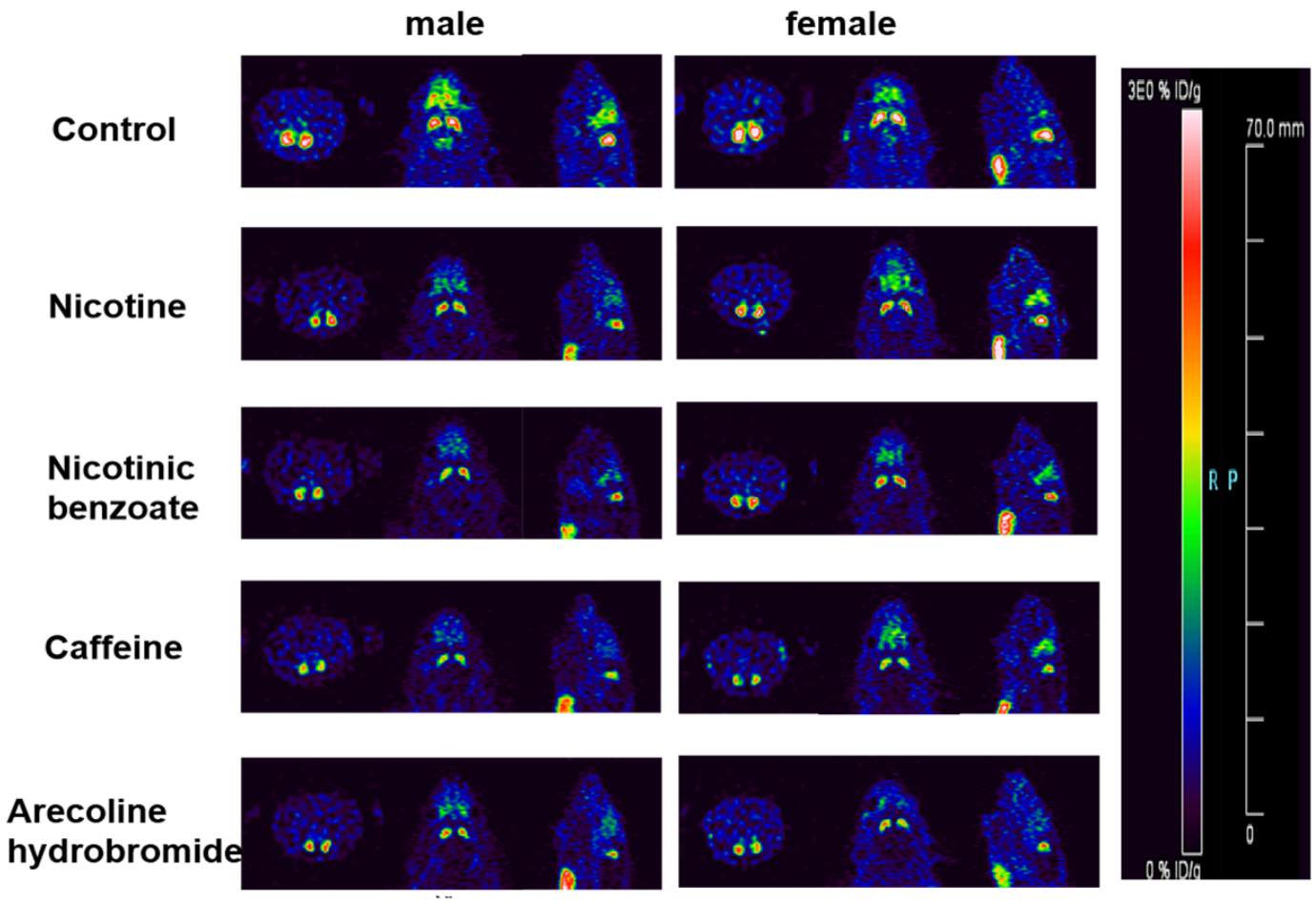

Figure 7

Distribution mapping of ^{18}F-Fallypride in SD male and female rat striatum after gavage administration different alkaloids/salts with same doses (0.1 mg/kg)

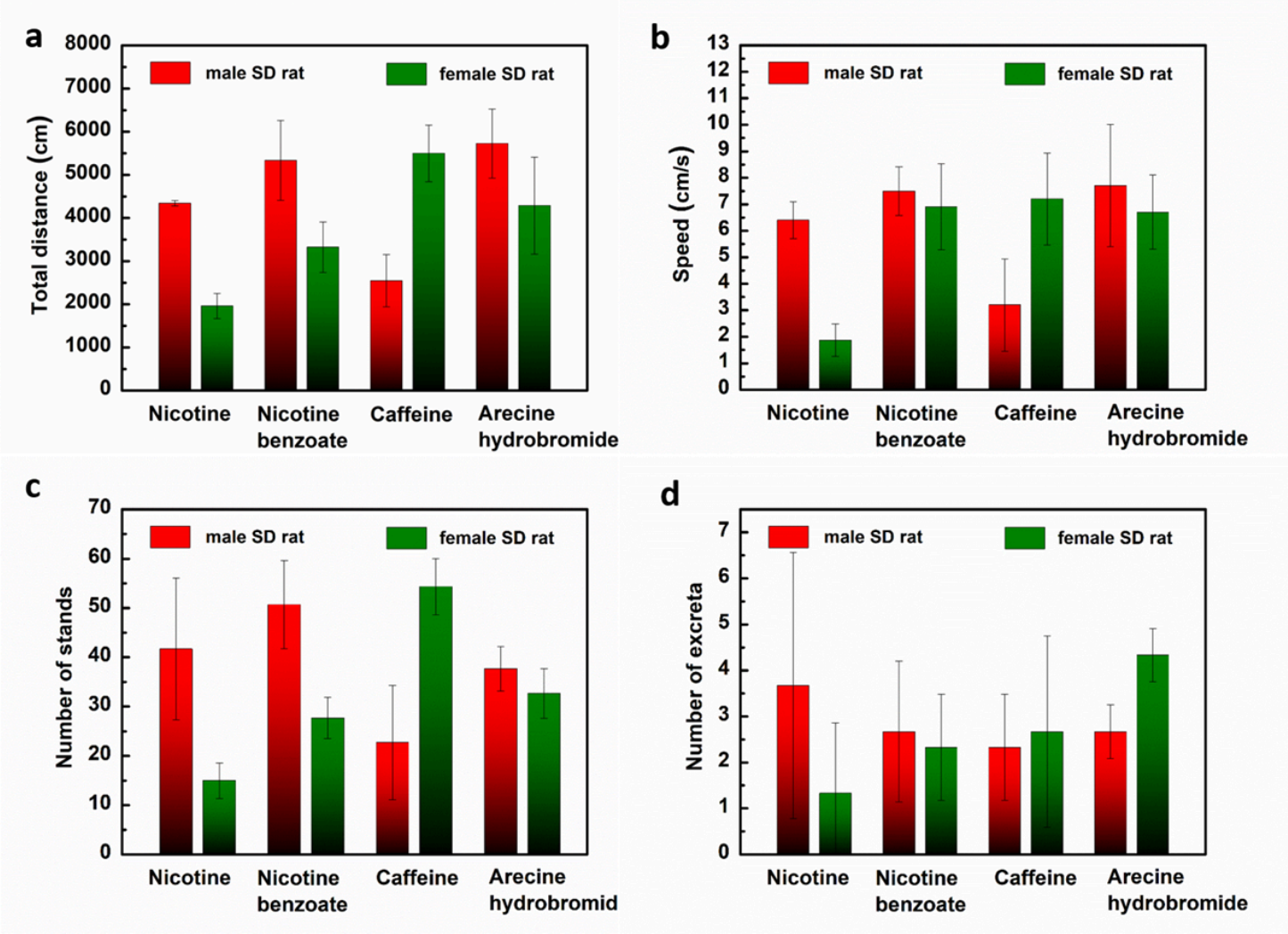

Figure 8

Behavior of male and female SD rats given the same dose of different alkaloids/salts