Neuroscience Letters 476 (2010) 27–31

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Acquisition and performance in a rat sequential reaction time task is not affected by subtotal ventral striatal 6-OHDA lesions

M.T. Eckart*, M.C. Huelse-Matia, D. Loer, R.K.W. Schwarting

Philipps-University of Marburg, Department of Psychology, Experimental and Physiological Psychology, Gutenbergstraße 18, D-35032 Marburg, Germany

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 20 March 2010
Accepted 30 March 2010

Keywords:
Striatum
Serial reaction time task
6-OHDA
Dopamine
Instrumental behaviour
Rat

ABSTRACT

Based on findings of experiments with humans, non-human primates and rodents, it is commonly accepted that dopaminergic basal ganglia processes play a crucial role in procedural and sequential learning. Primal evidence for this hypothesis came from serial reaction time tasks (SRTT) studies, demonstrating that healthy controls show increased reaction times when visual stimulus presentation switches from a previously learned sequence to random stimulus presentation. This so-called interference effect was reduced in patients with Parkinson's disease. Since ethical and methodical aspects limit neurobiological research in human subjects, we developed a rat version of the human SRTT, which can be used to study experimentally induced brain damage. In the present experiment we investigated the effects of bilateral 6-OHDA lesions of the ventral striatum on sequential learning. The lesions led to subtotal dopaminergic depletions in the ventral striatum (58–60%) and also minor depletions in the medial neostriatum (32–46%). These lesions impaired task acquisition only moderately and did not worsen sequential performance since lesion and control animals showed a comparable interference effect when the trained sequence was tested against random stimulus presentation or violated sequences. In contrast, in an earlier SRTT experiment with medial neostriatal dopaminergic lesions (58–66%), the lesion animals were clearly impaired in their sequential learning as compared to controls. Therefore, we assume that subtotal dopamine loss in the medial neostriatum, rather than the ventral striatum, has a substantial effect on sequential learning.

The critical test in the present experiment is a food reinforced rat version (introduced by Domenger and Schwarting [7–10]) of the human SRTT, which was first introduced by Nissen and Bullemer [15]. Analogous to the human SRTT, it is a four-choice task in which nose poking into illuminated holes accords to human key pressing.

In humans, the SRTT has commonly been used to investigate the role of basal ganglia dopamine (DA) in sequential behaviour since some clinical studies indicated that patients with Parkinson's disease (PD) showed impaired SRTT performance (mostly assessed in terms of reaction time (RT), e.g. [23]). Some studies did not replicate these findings (for discussion see Werheid et al. [24]), and it was claimed that these inconsistencies might be due to heterogeneity of clinical populations and treatment conditions. Thus, models are widely accepted, which propose the involvement of DAergic basal ganglia processes for the SRTT [13,11,6,22]. Work in rats has shown that especially the medial part of the neostriatum may be crucial for sequential learning [12], while the ventral striatum/nucleus accumbens is more relevant for stimulus-reward learning [17], or generally for basic Pavlovian and instrumental conditioning [1].

In an earlier SRTT study on sequential performance [7], bilateral neostriatal 6-hydroxydopamine (6-OHDA) lesions were placed after rats had been trained on sequences. Such striatal 6-OHDA lesions permanently damage the DAergic neurons and are a common early stage model of PD in rodents [2]. We chose an early stage model of PD, because there is evidence, that deficits in implicit memory already occur in pre-symptomatic stages of PD [16] (for similarities between human and mammalian basal ganglia involvement in implicit learning see Doyon and Ungerleider [11]). Regarding sequential behaviour, animals with such DA lesions conducted less automated responses and did not retain their superior sequential RT performance, whereas they preserved their level of performance in terms of accuracy. Furthermore, the lesions were not sufficient to completely abolish sequential performance, but these animals were unable to further improve their SRTT performance after surgery while controls did. This result hints at a role for neostriatal DA in learning as compared to established performance. This hypothesis was supported by a recent experiment in which the neostriatum was lesioned by 6-OHDA prior to SRTT training [12]. These lesions resulted in subtotal DA depletions of 58–66% in the medial neostriatum. They led to deficits in sequential learning in terms of RT and response accuracy when compared to performance under random stimulus presentation.

* Corresponding author. Fax: +49 6421 2826621.
E-mail address: eckart@staff.uni-marburg.de (M.T. Eckart).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.03.075

Unlike the neostriatum, we know of no experimental evidence for an involvement of the ventral striatum (including the nucleus accumbens) in sequential learning and performance. Cole and Robbins [4] found only minor effects of 6-OHDA lesions of the rat nucleus accumbens on performance in the 5-choice task, which is a serial but not a sequential one (see Schwarting [22]). Therefore, the present experiment was performed to test, whether bilateral ventral striatal 6-OHDA lesions affect sequential learning and performance in our rat sequential task. Since we had no explicit hypothesis concerning specific parts of the ventral striatum (like nucleus accumbens core or shell) we aimed at the whole lower third of the striatum around the nucleus accumbens (termed ventral striatum). After surgery, the animals underwent the same training and testing schedule as in the previous neostriatal lesion study [12]. First, the animals were trained in the SRTT on a 12-item sequence. Thereafter, two tests of sequential learning were applied. First performance during phases of sequential stimulus presentation was compared against random presentation (S-R-S-R test; [10]). Typically, rats show increased reaction times and less response accuracy under random conditions. In the second test (sequence violation test), the animals were run under sequential stimulus presentation [7], but after several minutes, single sequence items were violated. Typically, reaction times are increased to such violated items. Also, rats show indices of response automation since they often respond to the sequentially "expected" item.

Thirty male Wistar rats (Harlan-Winkelmann, Germany), weighing 250–274 g at the start of the experiment were used. They were kept under a 12:12 h light/dark cycle, housed singly with ad libitum access to water.

Surgery was performed under xylantine (Rompun®, Bayer, 0.5 ml/kg) and ketamine (Ketavet®, Pharmacia, 1 ml/kg) anaesthesia. The lesion group ($n = 20$) received injections of 1 μl of 6-OHDA per injection site (concentration: 16 μg/μl), dissolved in an ascorbate solution (1.0 mg/ml in 0.9% saline) into the ventral striatum via a Hamilton injection pump (Hamilton Co., USA) at a rate of 0.35 μl/min. The coordinates (from bregma) were: anterior: +1.6; lateral: ±1.8; ventral: −7.4 from dura according to Paxinos and Watson [18]. After injection, the cannula remained in place for further 5 min. Controls ($n = 10$) underwent the same procedure, but received only the ascorbate solution. After surgery, the animals were given 1 week for recovery.

We used the same experimental schedule as in the neostriatal lesion study [12]. All tests and apparatus were described in detail there. Thus, we only give a short description of the order of the experimental schedule:

- *Catalepsy test*: This test was used to scan the animals for gross motor deficits. The animals were placed with their forepaws on a rectangular plastic block (4.5x14x12.5 cm) and the time was measured until the animal moved its paws.
- *SRTT*: Before starting the SRTT, rats had to be food-deprived. They were kept between 80 and 85% of their free feeding weight. For SRTT testing (for details see [7–10]), we used modified operant chambers (MedAssociates) with four LED-equipped holes (i.e. nosepoke holes) arranged in a small alcove in a semi-elliptic way tilted towards the pellet-receptacle. The holes where numbered as: [1] upper left, [2] upper right, [3] bottom left, [4] bottom right. The pellet-receptacle was connected to a dispenser, which delivered adjustable amount of pellets (dustless precision pellets, 45 mg each, Bioserve, Bilaney Consultants, Germany). Basically, the rats had to respond to visually stimuli by poking into the illuminated (i.e. active) hole. After a correct response, the light was immediately lit in another hole. The order of the illuminated holes was either pseudo-random (i.e. the same hole was never lit twice in a row) or followed a second-order 12-item sequence (in each case two consecutive items predict the following item: 3-2-4-1-3-4-2-1-2-3-1-4 [19]). Responding was reinforced on a fixed ratio schedule of 13 (FR 13), that is, reward was delivered after every 13th correct poke. This ensures that sequence and reinforcement are dissociated (single items of the sequence cannot be associated with reward delivery).
  - *Basic training*: The animals were shaped daily (20 min each) until they reached the criterion of FR 13. Finally, a 5 s time limit for poking was applied. The active hole was illuminated until the rat poked (correct or incorrect) or until the time limit had expired (omission). In case of omissions and wrong pokes, feedback was provided by a tone and house light illumination. Then, stimulus presentation continued from that item, where the sequence had been interrupted [12].
  - *SRTT training*: Using the FR 13, the rats were trained daily (20 min each) on the sequence until the lesion group showed a stable level of performance in terms of no significant RT improvements over 3 consecutive days.
  - *S-R-S-R test*: Here, test conditions alternated between sequential and random stimulus presentation (min 1–5: seq_1, min 6–10: $rand_1$, min 11–15: seq_2, min 16–20: $rand_2$). The time limit for responding was 5 s, but the active hole was only illuminated for 1 s.
  - *Sequence violation test*: In the first 5 min of this test, the stimuli were presented in the trained sequential order. Afterwards, approximately 20% of the sequences were violated by skipping one item of the sequence on the 9th position of the FR. By linking the violations to the FR, the errors occurred on different positions of the sequence. We analysed the effects of the violation on the reaction time and the type of pokes made at the violated positions. These pokes were categorised as: correct (into the illuminated hole), expected (into the skipped hole according to the trained sequence), and random errors (into any other hole).

The animals were decapitated under deep anaesthesia (pentobarbital-sodium, Narcoren®, Merial, Germany, 2.5 ml/kg) and the brains were immediately removed. The striata of both hemispheres were extracted and divided into three parts: ventral striatum, lateral neostriatum and medial neostriatum. Tissue samples were weighed in 0.05 M percloric acid, sonicated, centrifuged and stored at −80 °C. They were analysed for their contents of DA and serotonin using high performance liquid chromatography with electrochemical detection as described before [12].

For the calculation of RTs, only correct pokes (i.e. into illuminated holes) were taken into account. For accuracy, we calculated the percentage of correct pokes from the total pokes (correct and wrong pokes). All results are expressed as mean ± S.E.M. Analyses were done using SPSS 17.0. All data were checked for normal distribution by Kruskal–Wallis tests. Since they fitted the normal distribution, only parametric tests were applied, i.e. ANOVAs and SIDAK-corrected *t*-tests.

Compared to controls, ventral striatum DA in the lesion group was significantly depleted, namely by 59.9% ± 7.98 in the right ($t_{25} = -4.33$; $p < 0.01$) and by 57.94% ± 7.78 in the left hemisphere ($t_{25} = -4.42$; $p < 0.01$).

In the medial neostriatum, the corresponding values were 31.95% ± 11.89 right and 46.41% ± 11.18 left, but depletion was significant only on the left side (right: $t_{27} = -1.62$; $p = 0.12$; left: $t_{27} = -2.27$; $p = 0.03$).

In the lateral neostriatum, DA depletions were not significant (right: 16.34% ± 11.34, $t_{27} = -0.84$; $p = 0.409$; left: 6.68% ± 11.72, $t_{26} = -0.29$; $p = 0.77$).

There were no group differences in serotonin in any of the structures (data not shown).