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Impaired pupillary control in "schizophrenia-like" WISKET rats

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ABSTRACT

Patients with schizophrenia show impairments in autonomic regulation, including pupillomotor control. The aim of this study was to explore the changes of pupillary light reflex in a new substrain (WISKET) with several schizophrenia-like alterations.

Male WISKET rats housed individually (for four weeks) and treated with ketamine (for 3 × 5 days) after weaning and naive group-housed Wistar rats (controls) were involved in the study. The pupillary light reflex was studied in two series after sedation (diazepam) or anesthesia (chloral hydrate). Video recordings were evaluated with custom made video analyzer software.

Several significant changes were observed between the two groups: the initial and minimum pupil diameters were greater, the degree of the constriction was lower, and the flatness of the curve and the total duration of constriction were shorter in the sedated WISKET rats. No other pupillary parameters (latency, amplitude and redilation) showed significant alterations.

Chloral hydrate anesthesia prolonged the constriction and redilation processes compared to the sedated animals, and diminished the differences between the groups.

In conclusion, WISKET rats showed disturbances in the pupillary light reflex, suggesting a general shift of autonomic balance towards a sympathetic predominance. The results provide further evidence to support the validity of WISKET rats as a complex, chronic animal model of schizophrenia.

1. Introduction

Patients with schizophrenia show not only behavioral impairments but also autonomic dysregulation, manifest as decreased variability of blood pressure and heart rate, abnormal thermoregulation and impaired pupillary function (Bar et al., 2007, 2008; Hermesh et al., 2000; Rubin and Barry, 1972; Shiloh et al., 2005, 2007; Zahn and Pickar, 2005). Pupil responses are sensitive and reliable sources of information about the function of the nervous system, including its autonomic division. Pupillometry, a simple, non-invasive technique, can be utilized for the objective characterization of the pathophysiology of pupillary functions, and it has long been applied in human diagnostics (Bremner, 2009; Neuhuber and Schrodl, 2011). Pupil diameter is determined by two antagonistic smooth muscle groups, the sphincter and dilator muscles within the iris. Pupillary constriction or miosis is brought about by the action of the sphincter muscle, whereas pupillary dilation or mydriasis happens by the contraction of the dilator muscle. The sphincter muscles receive primarily postganglionic parasympathetic fibers from the oculomotor nerve through the ciliary ganglia, and the preganglionic fibers originate from the Edinger-Westphal (EW) nucleus located in the midbrain. Generally, the parasympathetic pathway provides a tonic drive to the sphincter iridis muscle through the activation of M3 muscarinic receptors, and their enhanced activation causes miosis. The dilator muscles receive noradrenergic sympathetic input from the superior cervical ganglion, which acts primarily on α1-adrenoceptors; providing only little tonic drive. Thus, pupillary function is determined by a balance between the sympathetic and parasympathetic autonomic nervous system (ANS), such as reciprocal control, co-inhibition or co-activation which depend on a number of factors, including genetic influences, age, wakefulness, accommodative state and ambient lighting conditions (Neuhuber and Schrodl, 2011). Therefore, the resting pupil size may vary over a wide range even in healthy individuals (Bremner, 2009; Goldwater, 1972).

The pupillary light reflex (PLR) is a primitive, cross-species, bidirectional

Abbreviations: ANS, autonomic nervous system; ASD, autism spectrum disorder; C, control; EA, exploratory activity; EW, Edinger-Westphal; LC, learning capacity; PA, pulse alone; PLR, pupillary light reflex; PP, prepulse - pulse pair; PPI, prepulse inhibition; TF, tail-flick; W, WISKET

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reflex. Under normal circumstances, bright light shone into one or both eyes produces a brisk and transient contraction followed by a slow redilation (Goldwater, 1972). The reflex is mediated by a well-characterized and relatively simple neural circuit, involving the retinal ganglion cells, the pretectal nuclei, the mesencephalic EW nucleus, the ciliary ganglion, and the pupillary sphincter muscle (Neuhuber and Schrodl, 2011).

Those clinical studies that used pupillometry to investigate the autonomic function in schizophrenic patients yielded controversial results, but most of them suggested an increased sympathetic modulation and/or decreased parasympathetic activity (Bar et al., 2008; Hakerem et al., 1964; Lidsky et al., 1971; Morris et al., 1997; Okada et al., 1978; Rubin and Barry, 1972; Steinhauer and Hakerem, 1992; Steinhauer et al., 1992). The physiology, pathophysiology and pharmacology of pupillary functions were also investigated in rodents (Mohan et al., 2012; Young and Lund, 1994), however, no data on pupillary function are available from animal models of neuropsychiatric disorders, including schizophrenia.

Recently a complex, chronic rat substrain of schizophrenia has been derived, that shows several symptoms of the disease. The new substrain was named **WISKET**, since the original strain was **WIS**tar and the selective breeding was based on behavioral alterations after the combination of postweaning **IS**olation rearing and subchronic **KET**amine treatment (Horvath et al., 2016, 2017; Kekesi et al., 2015; Petrovszki et al., 2013). These animals exhibit disturbances in sensory gating and pain sensitivity, altered auditory evoked potentials, and impairments in various cognitive functions. Furthermore, the binding affinity of opioid and cannabinoid receptors in the brain of these animals is also significantly different from that of Wistar rats (Szűcs et al., 2016a, 2016b). In a recent study, we applied telemetric method for body temperature registration, and found that WISKET rats had higher body temperature during the active phase, and they showed a wider range of the body temperature alterations than the control Wistar rats, which suggests disturbed thermoregulation (Horvath et al., 2015). In the present study, we sought to further investigate the alterations of autonomic control in the new substrain by characterizing the changes of PLR.

It is well-known that the test procedure leads to a significant stress response in the animals, which can influence ANS functions. While anesthesia can prevent the stress and allows a convenient investigation of pupillary reactions for a longer period, it diminishes the autonomic responses (Hussain et al., 2009; Tayefeh et al., 1997). Therefore, the second goal of this study was to compare the pupillary responses in lightly sedated and anesthetized control and WISKET rats.

2. Materials and methods

2.1. Subjects

All experiments were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: XIV/2014). Animals were kept with a 12 h light/dark cycle under controlled temperature (22 ± 1 °C) with ad libitum water and food access (except for the experiments in the AMBITUS system, when the animals were food deprived for two days, see below, but water was always freely available).

2.2. Selective breeding

The paradigm for selective breeding, starting from a population of outbred Wistar rats, was described previously in detail (Petrovszki et al., 2013), and is shown in Table 1. Briefly, after weaning, at 3 weeks of age, the rats were tested with the tail-flick (TF) test to assess their basal pain sensitivity and then housed individually for 28 days (between 4 and 7 weeks of age). The animals in each generation were treated with ketamine (Calypsol, Gedeon Richter Plc., Budapest, Hungary; 30 mg/kg intraperitoneally [i.p.], 4 ml/1000 g body weight, daily, 5 times/week, 15 injections in total) from 5 to 7 weeks of age. Then the animals were re-housed in a group setting (3–4 rats per cage) and 1 week of recovery with no treatment followed. Starting at the age of 9 weeks, TF latency, sensory gating (pre-pulse inhibition), exploratory activity and cognitive functions were assessed (see below). Animals with the highest level of disturbance in these parameters were used for selective breeding throughout several generations (Horvath et al., 2015, 2016, 2017; Kekesi et al., 2015; Petrovszki et al., 2013; Szűcs et al., 2016a, 2016b).

2.3. The experimental paradigm

Two series of experiments were performed one week after the routinely executed behavioral tests in naive socially rearing/group-housed male Wistar rats without any interventions (Controls: C) and male WISKET (W) rats (Table 1) derived from multiple litters.

In the first series, the pupils were tested 15 min after diazepam-induced sedation (Seduxen, Gedeon Richter Plc, Budapest, Hungary; 2.5 mg/kg i.p.; W: n = 22, C: n = 17) for up to 15 s. The sedated animals had slow righting reflex and they accepted the slight restrain during the test period.

In the second series, the pupils were tested 15 min after chloral hydrate-induced anesthesia (Gedeon Richter Plc, Budapest, Hungary; 200 mg/kg i.p.; W: n = 20, C: n = 14) for up to 60 s (Lau et al., 1992). The anesthetized animals had no righting reflex and there was no response to mechanical stimuli.

The experiments were performed in each animal (in both series) between 8:30 and 12:30 without any training section.

2.4. Procedures

2.4.1. Nociceptive testing

Acute nociceptive threshold was assessed by the TF test at the 4th and 9th weeks of age (Table 1). The reaction time was determined by immersing the distal 5 cm portion of the tail in hot water (48 °C) until a tail-withdrawal response was observed (cut-off time: 20, 40 s, on the 4th and 9th weeks, respectively). TF latencies were obtained four times at 30 min intervals and were averaged to establish the pain threshold for each group.

2.4.2. Sensory gating testing

The degree of sensory gating (PPI) of the acoustic startle response was measured as described previously (Petrovszki et al., 2013). Briefly, after 10 min habituation in plexiglas startle chambers (12 × 17 × 15.3 cm) rats were exposed to two different trial types: the pulse alone (PA), in which a 40 ms 95 dB white noise burst was presented; and the prepulse - pulse pair (PP) in which prepulse stimuli (20 ms, 76 dB) were followed by the startle stimulus with a latency of 150 ms. Both types of stimuli were applied 20 times in random pattern. The interstimulus intervals ranged from 7 to 13 s. PPI was calculated as percentages using the following equation: PPI (%) = [1 − (*startle response for PP*) / (*startle response for PA*)] × 100.

2.4.3. Appetitively motivated cognitive task in the AMBITUS system

The AMBITUS system is a cognitive-behavioral test suitable for the detection of exploratory activities and learning capacity in rats (Horvath et al., 2017). Briefly, it is a square corridor of clear plexiglas on black floor (outer diameter of 80 cm, width of 8 cm and height of 50 cm), and all the eight walls have two equally spaced sites (side-boxes: 5 × 5 × 5 cm) with food reward in each of them (puffed rice, 20 mg) and equipped with infrared photocells for the detection of the exploratory activity (visits into the side-boxes) of the animals. The tests were also recorded using an infrared video device (WCM-21VF, CNB, China) fixed above the apparatus.

Trials commenced by placing the rats into the same starting point within the corridor; thereafter, the experimenter immediately left the room. The animals were allowed to explore the corridor and collect the 16 food rewards within 5 min (cut-off time). The number of food