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Quantitative evaluation of orofacial motor function in mice: The pasta gnawing test, a voluntary and stress-free behavior test

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HIGHLIGHTS

- The pasta gnawing test measures orofacial motor deficits.
- The pasta gnawing test is useful as an alternative to limb motor tests.
- The pasta gnawing test is useful to test progression of early onset disease models.
- The pasta gnawing test is stress-free and depends on voluntary behavior.

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ABSTRACT

Background: Evaluation of motor deficits in rodents is mostly restricted to limb motor tests that are often high stressors for the animals.
New method: To test rodents for orofacial motor impairments in a stress-free environment, we established the pasta gnawing test by measuring the biting noise of mice that eat a piece of spaghetti. Two parameters were evaluated, the biting speed and the biting peaks per biting episode. To evaluate the power of this test compared to commonly used limb motor and muscle strength tests, three mouse models of Parkinson's disease, amyotrophic lateral sclerosis and Niemann-Pick disease were tested in the pasta gnawing test, RotaRod and wire suspension test.
Results: Our results show that the pasta gnawing test reliably displays orofacial motor deficits.
Comparison with existing methods: The test is especially useful as additional motor test in early onset disease models, since it shows first deficits later than the RotaRod or wire suspension test. The test depends on a voluntary eating behavior of the animal with only a short-time food deprivation and should thus be stress-free.
Conclusions: The pasta gnawing test represents a valuable tool to analyze orofacial motor deficits in different early onset disease models.

1. Introduction

Measuring motor behavior in rodent disease models is often performed in long lasting tests with time consuming 'paper pencil' evaluations and under high stress conditions, e.g. in the RotaRod or challenging beam walk test. Especially testing of rodent models with a severe phenotype or increased probability of epileptic seizures sometimes leads to insufficient results due to incapability of the animals to perform the task. In 2011, Kane and colleagues noticed that while performing a pasta handling test (Vermicelli and Capellini handling test) for evaluation of the lesion rate of unilaterally 6-OHDA injected rats, not only the number of adjustments with each paw was altered, but also the biting noise changed (Kane et al., 2011).

We therefore developed the voluntary pasta gnawing motor test for mice with a minimum of experimenter's or equipment interference and thus a minimum of stress for the animals. Since eating displays a basal natural behavior and biting noise can also be easily recorded from a distance, we established quantitative analyses of this behavior. Additionally, we analyzed if the orofacial motor test represents a good alternative to the most commonly performed evaluations of limb associated motor tests.

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To analyze the suitability and value of the pasta gnawing test, we thus measured the biting behavior compared to well-established limb motor tests in three mouse models representing three different indications and already known to present limb motor deficits: I: Line 61 mice overexpress α-synuclein and are a model of Parkinson's disease [PD (Rockenstein et al., 2002)], a disease that is, next to shaking and gait disturbances, also characterized by difficulties in mastication and orofacial function which can be observed in moderate to advanced disease stages (Bakke et al., 2011). Swallowing disturbances can already manifest in early and mid-stage PD (Jones and Ciucci, 2016). II: homozygous TAR6/6 mice overexpress TARDBP (TDP-43) and are a model of amyotrophic lateral sclerosis [ALS (Wils et al., 2010)]. About 70% of ALS patients with spinal disease onset suffer from dysarthria and dysphagia (da Costa Franceschini and Mourao, 2015), though patients with bulbar ALS are generally more severely affected compared to patients with corticobulbar or spinal ALS (Langmore and Lehman, 1994). These disturbances depend on muscle weakness in orofacial muscles, specifically the tongue (DePaul et al., 1988; DePaul and Brooks, 1993). Dysphagia is already measurable at the initial diagnosis of the disease (Murono et al., 2015) and thus a very early symptom. III: NPC1$^{-/-}$ knockout mice are a model of Niemann-Pick disease type C1 [NPC1 (Loftus et al., 1997)] that belongs to the lysosomal storage diseases exhibiting neurological symptoms like unsteady gait, tremor and progressive dementia, but also dysphagia and dysarthria (Vanier, 2010). About 80% of NPC1 patients suffer from dysphagia (Garver et al., 2007), while in the adult form of the disease only 37% present dysphagia and 63% dysarthria (Sevin et al., 2007).

2. Materials and methods

All animals were bred and housed under identical conditions in individually ventilated cages on standardized rodent bedding (Rettenmayer®) in the AAALAC accredited animal facility of QPS-Austria. Cotton nestlets (Plexx®) were provided as nesting material. The room temperature was kept at approximately 24 °C and the relative humidity between 40 and 70%. Mice were housed under constant light-cycle (12 h light/dark). Dried pelleted standard rodent chow (Altromin®) and normal tap water were available to the animals *ad libitum*. Each individual animal was checked regularly for any clinical signs. Only male animals were used. Mice were housed in same sex groups of up to four animals. During weaning, less than 1 mm of the tail tip was cut from each animal and used for genotyping. Behavioral tests were always performed in the morning during the light cycle. Before the start of each behavioral test, animals were habituated to the experimental room for at least 1 h. Age groups were chosen according to the observed phenotype onset (first age group) and late stage phenotype (last age group) of each mouse model and thus represent relevant time points for possible compound tests. Behavioral tests were performed in the order as mentioned below. Animal studies complied with the ARRIVE guideline (Kilkenny et al., 2010) and the Austrian guidelines for the care and use of laboratory animals and were approved by the Styrian government, Austria.

2.1. Line 61 mice

Line 61 mice express human wildtype α-synuclein under control of the murine neuronal Thy-1 promoter. Compared to endogenous α-synuclein, the transgene is about 10-fold higher expressed (Rockenstein et al., 2002). Animals are a commonly used model of Parkinson's disease. Heterozygous mice were bred by pairing one heterozygous male with two non-transgenic (ntg) females or by pairing one ntg male with two heterozygous females and the heterozygous offspring was tested compared to ntg littermates. Only male animals at the age of 8, 12 or 24 weeks were tested cross-sectional.

2.2. TAR6/6 mice

TAR6/6 mice express the human wildtype TARDBP (TDP-43) under control of the murine neuronal Thy-1 promoter. In homozygous TAR6/6 mice the TARDBP protein concentration is about 3.8-fold higher compared to endogenous TARDBP (Wils et al., 2010). Animals are a commonly used model of amyotrophic lateral sclerosis. Homozygous mice were bred by pairing one heterozygous male with one heterozygous female and homozygous offspring was tested compared to ntg littermates. Only male animals at the age of 6 and 20 weeks were tested cross-sectional.

2.3. NPC1$^{-/-}$ mice

NPC1$^{-/-}$ mice have a spontaneous mutation in the Niemann-Pick type C1 gene (NPC1^{m1N}). Animals homozygous for the mutation show decreased sphingomyelinase and glucocerebrosidase activity and are thus a commonly used model of Niemann-Pick disease (Loftus et al., 1997). Homozygous mice were bred by pairing one heterozygous male with one heterozygous female and offspring was tested compared to ntg littermates. Only male animals were longitudinally tested at the age of 6 and 8 weeks.

2.4. Pasta gnawing test

The test was adapted from (Kane et al., 2011). Two hours prior to testing the food of all animals was removed and animals were single housed. One little piece of dry spaghetti (Goldmarke, Spaghetti No. 5) was given to each animal to become familiar with the novel food in the moment the regular food was removed. To measure biting noise the home cage was placed in a sound proof cabinet and each animal was recorded at a time by placing a microphone above the cage. Dry spaghetti pieces (approx. 1 cm long) were given into the cage (Fig. 1A). Afterwards, the biting noise was recorded for one minute and an interval of ten seconds was evaluated. During the pasta gnawing measurement, all animals were observed by an experimenter, to guarantee that the sound recording was taken while the animal was indeed eating the pasta. Acquisition was performed by using Behringer ECM 8000 microphone connected to a Steinberg Cl1 audio interface. Steinberg Wave Lab LE 7 was used as recording software. The acquired biting pattern was analyzed using Avisoft SASLab Pro 5.1 sound analyzing software (Fig. 1B, C). When analyzing the sound recordings, the experimenter additionally checked the sound quality acoustically by listening to the sound sequence with a headset to clearly identify biting events. Afterwards an intensity threshold line (see Fig. 1) was set to separate biting events from background noise. Two parameters were evaluated: I: biting speed (biting frequency; bites per second within a biting episode) and II: biting peaks per biting episode (number of bites during a biting episode). An exemplary video showing a pasta eating mouse is provided in Supplementary file 1. The acoustic file related to this video recorded with the Behringer ECM 8000 microphone is provided in Supplementary file 2. The corresponding waveform and spectrogram of the start of the video analyzed by Avisoft software is thus shown in Supplementary file 3.

2.5. Wire suspension test

The test evaluates the muscle strength of mice. A standard wire cage lid was used. The mouse was placed on the top of the lid. Afterwards, the lid was slightly shaken to cause the mouse to grip the wires, and then turned upside down. Duct tape placed around