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Feasibility of repeated testing for learning ability in juvenile primates for pediatric safety assessment

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ABSTRACT

Assessment of learning ability in nonhuman primate (NHP) models is sometimes requested by regulatory authorities. The double choice object discrimination task using a Wisconsin General Testing Apparatus (WGTA) approach is typically being applied. In this study, the WGTA approach was performed on 66 juvenile cynomolgus monkeys aged 8–9 months in the predose phase of juvenile toxicity assessment. In addition, reversal learning data of seven control animals/gender were obtained for the weeks 25 and 52 of dosing. Gender differences in the number of days required to pass the habituation, learning or reversal learning phases were statistically comparable, males and females may be combined for statistical analysis. At first instance, the habituation phase was passed on average after 6.4 days, and the learning test on average after 8.6 days with improvement to 2.0–2.6 days for habituation and 6.4–6.7 days for learning in weeks 52. Power analysis ($\alpha = 0.05$, one-sided *t*-test) revealed a sample size of 8 and 41 to predict a 50% and 20% difference, respectively. In conclusion, examination for learning ability, but not for memory ability (during repeated testing) is feasible in juvenile NHPs using the WGTA approach.

1. Introduction

The availability of guidances when developing compounds with desired or potential pediatric indication has led to an increased need for the conduct of safety assessment in juvenile animals across several species. Mostly these studies are performed using rodent models (rats and mice), but in some cases, nonhuman primate (NHP) models have also been used. The choice of species is basically driven by the same considerations as for general safety assessment in that only relevant species should be used (Baldrick, 2013; Barrow et al., 2011; Leconte et al., 2011; Morford et al., 2011). Recent analyses suggest that the demand for juvenile toxicity testing in NHPs could be increasing (Weinbauer and Korte, 2015). Testing for learning ability is an important endpoint in pediatric safety assessment, especially if the clinical drug target is the (central) nervous system. For rodents, water mazes at various complexity levels are typically used to assess learning and memory capacities (Bailey et al., 2009). For NHPs – owing to their phylogenetic proximity to humans – the highly evolved behavioral repertoire of NHPs renders them highly valuable animal models for studies of the functional effects of potential drug therapies and neurotoxicants (Sibal and Samson, 2001; Burbacher and Grant, 2000).

However, validated approaches for assessing cognitive disturbances for neurotoxic evaluation of test items in NHPs are scarce. It has been shown that discrimination learning and reversal learning tasks are appropriate to study functional effects of neurotoxicant exposure (review: Burbacher and Grant, 2000) and the two-object discrimination and reversal task has been used successfully for testing cognitive performance in NHP models (Golub et al., 2005, 2007; Sackett et al., 2006; Mandell and Sackett, 2009). Discrimination and reversal learning tasks were used to examine neurotoxicity after developmental exposure to lead and TCDD (Bushnell and Bowman, 1979; Schantz and Bowman, 1989). The neuronal mechanisms underlying the formation of stimulus–reward associations and the ability to reverse these associations are well known (Rolls, 2000; Schultz and Dickinson, 2000; Clark et al., 2004). For the Wisconsin General Testing Apparatus (WGTA), which has regulatory acceptance in the absence of validated alternative approaches (Cappon et al., 2012), it was reported, however, that the inter-animal variance is quite high, meaning a large group size will be required to reach statistically significant results. This became evident from power analysis described by Cappon et al. (2012)

Abbreviations: WGTA, Wisconsin General Testing Apparatus; TCDD, 2,3,7,8-Tetrachlorodibenzo-p-dioxin; NHP, Nonhuman primate.

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using WGTA testing to assess the cognitive impairment in infant cynomolgus monkeys in enhanced pre- and postnatal developmental (ePPND) studies. It was reported that with $\alpha = 0.05$ and a one-sided t-test, a group size of seven animals provided 80% power to predict a 100% increase, whereas a group size of 25 would be necessary to predict a 50% increase. In addition, there is conflicting information whether there is a gender difference in performance in the WTGA or whether data of both genders can be combined for statistical analysis which could help to increase the statistical power. In fact, contradictory findings have been reported. Two studies failed to detect a gender associated effect in six months old or adult cynomolgus monkeys (Makori et al., 2013; Bachevelier et al., 1990). However, in animals of younger age, single gender differences were observed, such as a better performance of 3 months-old rhesus females in comparison to age matched males (Bachevalier et al., 1989, 1990; Mandell and Sackett, 2009) or a better performance of 75 days old males in comparison to females (Goldman et al., 1974).

Here we report our experience using the WGTA in 66 juvenile cynomolgus monkeys in the context of a regulatory pediatric safety assessment study. Animals were only exposed to vehicle treatment. Unlike in previous studies and reports, we had the opportunity to re-test some animals on two further occasions (once at baseline and twice during the dosing period) throughout a period of over 52 weeks. Interestingly, during repeated testing intervals approx. six months apart, only examination for learning ability but not for memory ability was possible using the WGTA approach.

2. Material and methods

2.1. Animals

Sixty-six juvenile cynomolgus monkeys (33 animals/gender), captive bred at NafoVanny, Vietnam, were used in this study. The study was conducted at Covance Preclinical Services GmbH, Muenster, Germany. All animals were weaned at six months of age at the breeder and kept in groups of weaned animals until transport. Upon arrival at the test site, the animals were quarantined for at least 4 weeks and were 8–8.5 months of age and weighed 1.1–1.8 kg at start of the first learning test. During the predose phase of the study, animals were housed in standard ETS 123-compliant (ETS 123, Appendix A: Guidelines for Accommodation and Care of Animals, 18 June 2007) cage systems with 6 animals/cage and sex (Müller, 2008). The cages are made of stainless steel and have a height of 248 cm, a width of 151.5 cm/151 cm, a floor space of 2.29 m^2 and a volume of 5.68 m^3 (plus a balcony). For dosing start, animals were separated in smaller groups of 2 or 3 animals/cage and sex. The animal room was maintained at a temperature of 19–25 °C and a relative humidity of 40–70%. Animals were kept on a 12:12 light schedule (white light illumination switched on at 5.30 am). The monkeys were fed a standard monkey diet and UV-irradiated and filtered tap water was provided to the animals *ad libitum*. The monkeys were provided environmental enrichment (wooden chips, movable stainless steel mirrors, colored plastic tools and colored plastic balls). The health status of all animals was monitored daily. In this pediatric safety study, animals received treatments throughout a period of up to 52 weeks. For WGTA tests, all study animals were studied during the baseline period. For the treatment phase, this data description is confined to control animals – these animals received weekly subcutaneous injections of saline up to 52 weeks. The study was conducted in accordance to the German Animal Welfare Law and was reviewed and assessed by the Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental setup of the WGTA

Behavioral testing took place in a modified Wisconsin General Test Apparatus (WGTA; Makori et al., 2013; Bachevalier and Mishkin, 1984, Fig. 1) located in a noise-reduced and dark room.

The double choice object discrimination task is an operant learning test, meaning the animals have to operate objects in their environment to obtain a reward (in this case food such as Fruit loops or Smarties). Operant tasks always require some degree of training; furthermore, to guarantee motivation of the animals, access to food needs to be restricted prior to the test. The animals were fasted 2.0–3.5 h before testing started. They were transferred from their home cage into a white plastic transport box. The animal containing transport box was brought to the testing room and connected to the WGTA (A). The front of the small box consists of a clear acrylic glass with two round holes where the animal can reach through. A panel was placed on the top front of the transport box which could be raised and lowered to allow access to the stimulus tray (B). It was located in front of the cage inhibiting the animals visibility range outside the box, thus hindering the animal to watch any actions in front of the box. The stimulus tray contained three shallow wells, on the right, in the middle, and on the left. Food rewards (Smarties, Fruit loops, raisins, grapes, pieces of apple, or small pieces of cucumber) were placed in the left or right well of the stimulus tray. The experimenter was hid behind a semi-reflecting mirror (C) leaving illuminated gap in the apparatus for the behavioral animal testing. Testing was performed daily. Furthermore, change in personnel as experimenter or animal care taker was restricted to a minimum.

In the habituation phase, the experimenter placed a food reward in both wells of the stimulus tray. Much larger objects (cubes made of white styrofoam) were placed behind the wells letting the animal see the reward in front of the objects. The tester then raised the door to allow the monkey access to the stimulus tray. Each session consisted of 120 s and it was recorded whether the animal took the left and/or right food reward. Ten sessions were performed per day. Once the animal was trained to take the food reward, the wells were covered by the object and the monkey again was given 120 s to displace one or both of the objects and to obtain the food reward. The habituation phase was finished once the animal displaced the

Fig. 1. Wisconsin General Testing Apparatus (WGTA) – A: animal transport box, B: reward stimulus tray, C: semi-reflecting mirror.