The neuro-toxin MPTP does not prevent reproduction in marmosets [version 2; peer review: 2 approved]

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Abstract
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neuro-toxin that has been employed to model Parkinson’s disease in non-human primates for over 3 decades. Despite its use for such a long period, little is known about the effects of MPTP on reproductive function. Here, we report the case of a male marmoset which was able to procreate 1.5 year after having been administered the toxin. We also report on 1 male and 1 female MPTP-lesioned marmosets which produced babies after being housed together for 5 years. These cases suggest that MPTP may not interfere with marmoset reproductive function or that if it does, it may be for a limited period of time.

Keywords
MPTP, marmoset, reproduction

Open Peer Review

Reviewer Status ✔ ✔

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Any reports and responses or comments on the article can be found at the end of the article.
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Author roles: Nuara SG: Investigation, Writing – Review & Editing; Burgess LA: Investigation, Resources, Writing – Review & Editing; Hamadjida A: Investigation, Writing – Review & Editing; Gourdon JC: Investigation, Resources, Supervision, Validation, Writing – Review & Editing; Huot P: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: PH has received payments from UCB.

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Introduction

The common marmoset (Callithrix jacchus) has been used to model Parkinson’s disease since 1984 and since then, nearly 100 experimental drugs that aim at alleviating disease manifestations and treatment-related complications have been assessed in this small primate. Marmosets are typically rendered parkinsonian by administration of a neuro-toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which destroys dopaminergic neurons, leading to a parkinsonian phenotype.

Despite this abundant literature, whether MPTP interferes with marmosets’ ability to procreate has, to our knowledge, not been documented. Here, we report the case of 3 marmosets which were able to generate offspring after having been administered MPTP.

Methods

Animals

Two male and 1 female common marmosets (from McGill University breeding colony), weighing 350–400g, were pair-housed under conditions of controlled temperature (24±1°C), humidity (50%) and a 12h light/dark cycle (07:15 lights on). They were cared for in accordance with a protocol approved by McGill University and the Montreal Neurological Institute Animal Care Committees in accordance with the regulations defined by the Canadian Council on Animal Care. They had unlimited access to water and were fed with marmoset food (Mazuri®, catalogue number 0007066, USA), nuts and fresh fruits twice daily. Home cages were enriched with primate toys, nest-boxes and perches. All efforts were made to avoid causing harm to animals; we achieved this by working in close collaboration with the Animal Care Committees and veterinary teams.

Induction and assessment of parkinsonism

Parkinsonism was induced by s.c. injections of MPTP hydrochloride in the back of the animals (2 mg/kg every other day, for 5 days, at 08:30; catalogue number M0896, Millipore-Sigma, Canada), as previously reported. Animals were not sedated during the injections.

Following a 6-week recovery period, a stable parkinsonian phenotype was observed. For behavioural assessment, each marmoset was placed individually into an observation cage (36 × 33 × 22 in) containing food, water and a wooden perch, and left undisturbed for 6 h. Behaviour was recorded via webcam and analysed post hoc by a movement disorder neurologist blinded to the treatment given.

Parkinsonism was scored for 5 min every 10 min using a scale that combined measures of range of movement, bradykinesia, posture, and attention/alertness. Range of movement was rated on a 0 to 9 scale: 0 = running, jumping between roof, walls, perch, using limbs through a wide range of activity; 9 = no movement. Bradykinesia was rated from 0 to 3: 0 = normal initiation and speed of movement; 3 = prolonged freezing, akinesia, inability to move. Postural abnormalities were rated 0 or 1: 0 = normal balance, upright posture, head held up; 1 = impaired balance, crouched posture, head down. Attention/alertness was rated 0 or 1: 0 = normal head checking movements, movement of neck in variable directions, smooth, small movements; 1 = reduced or absent head checking, head in one position for more than 50% of observation period. The score attributed to each of the behaviours assessed was the most prevalent of the 5 min observation period. The global parkinsonian was calculated as a combination of the above behaviours according to the following formula: (range of movement × 1) + (bradykinesia × 3) + (posture × 9) + (attention/alertness × 9). The maximal parkinsonian disability score per 5 min observation period was 36.

Housing and breeding

As mentioned above, marmosets were pair-housed. One male marmoset was paired with a female marmoset which had not been exposed to MPTP, while 1 MPTP-lesioned male and 1 MPTP-lesioned female marmosets were housed together.

Behavioural assessment of the young marmosets

To minimise stress to families and to avoid interfering with the rearing process, newborn marmosets were not caught and all observations were made by experienced animal care technicians in the animal’s home cages.

Euthanasia

As we will report later, two newborn marmosets had to be euthanised. They were administered ketamine (Vetoquinol N.-A Inc, Canada) 20 mg/kg intra-muscularly, followed by 100 mg/kg sodium pentobarbital (Vetoquinol N.-A Inc, Canada) intravenously, according to McGill University standard operating procedures (https://www.mcgill.ca/research/research/compliance/animals/animal-research-practices/sop).

Results

Parkinsonism

Acute administration of MPTP resulted in the development of a parkinsonian phenotype, characterised by bradykinesia, hunched posture, reduced activity and reduced alertness in all 3 animals, as previously reported.

Pairing and breeding

Parkinsonian male marmoset paired with non-parkinsonian female marmoset. At the time of the pairing (May 2017), the male marmoset was 3.5 years old and he was paired with a 3-year old female marmoset. The female marmoset was pregnant at the time of the pairing and gave birth to twin babies (1 male and 1 female baby) 4 months later (September 2017). Despite not being the biological father of these 2 babies, the male marmoset was an active and attentive participant in the rearing of these two babies.

Five months later (February 2018), i.e. 9 months after the pairing, triplets were born, of which 2 survived. Based on
our experience, when female marmosets give birth to triplets, it is expected that one baby may not survive, and it is therefore unlikely that this casualty can be attributed to MPTP. Another 6 months later (August 2018), a second set of triplets, of which 2 survived, were born. More recently, twins were born (January 2019).

Thus, the male marmoset that was administered MPTP has so far fathered 8 babies, 6 of which have survived, are healthy and are developing normally, e.g. weaning, range of motion, body size, etc. In addition, the male marmoset has also been actively involved in the care and rearing of the babies.

**Parkinsonian male marmoset paired with parkinsonian female marmoset.** A male and a female marmoset, both aged ≈ 8 years old, were administered MPTP 5 years ago and have been pair-housed together since then. The female marmoset has been administered monthly injections of progesterone-based contraceptive. Quite unexpectedly, despite the parkinsonian state of both animals and the monthly injections, the female marmoset gave birth to twins in September 2018. Because experiments in which the female marmoset was administered investigational products were conducted during the pregnancy and that the impact of these substances on babies was uncertain, and also because the adequacy of the care provided by the 2 parkinsonian parents was uncertain, a decision was made to euthanise the newly-born marmosets shortly after their birth.

**Discussion**

Here, we report that marmosets were able to procreate after having been administered with the neuro-toxin MPTP. These cases suggest that MPTP, when administered acutely may not be toxic to the reproductive system, in the marmoset. Moreover, MPTP-exposed marmosets appear to be able to care for their offspring, despite their parkinsonism. It is noteworthy that marmosets are New World monkeys; it is currently unknown whether Old World monkeys, apes or humans would be able to procreate after exposure to MPTP.

In the case of the parkinsonian male marmoset paired with the non-parkinsonian female marmoset, that babies were born 9 months after the pairing occurred makes a strong case that the MPTP-exposed animal is the biological father of the offspring. The average gestation period of a female marmoset lasts 143-144 days, and twin babies are common; the birth of another set of triplets 5 months after the last delivery is an agreement with the duration and the outcome of a normal pregnancy, as is the subsequent birth of twins.

In the case of the paired MPTP-lesioned marmosets, that the 2 animals were housed exclusively together for years indicates that babies were conceived by 2 parkinsonian animals. It is nevertheless surprising that the female marmoset was able to become pregnant despite the monthly administration of contraceptive. It is possible that the injection was not optimally timed within her cycle; whether MPTP slightly interfered with the cycle duration remains uncertain.

In the brain, MPTP is converted to its toxic metabolite, 1-methyl-4-phenylpyridinium [MPP+] by the enzyme monoamine oxidase B [MAO-B] in astrocytes and then enters dopaminergic neurons via the dopamine transporter. The dopamine transporter is expressed, albeit at low levels, in the hypothalamus and MPTP administration was shown to produce degenerative changes to hypothalamic dopaminergic neurons, in the marmoset. This could, in theory, lead to disruption of the hypothalamo–hypophyso–gonadal axis with possible incapacity to procreate, but that does not seem to be the case, based on our report.

Several organs in addition to the brain express the MAO-B, notably blood, kidneys and liver, representing sites where MPP+ could be generated. However, the dopamine transporter may not be present within the testis, which is why MPP+ may not have led to a loss of reproductive function. Recently, it was shown that the dopamine transporter may be present on spermatozoids, suggesting that perhaps MPTP could be toxic to spermatozoids acutely, but with on-going spermatogenesis, this effect might be short-lived.

MPTP could also be toxic to the autonomic nervous system in rhesus macaques, but a recovery process seems to happen.

In summary, this report suggests that MPTP administration may not affect the reproductive function in marmosets or, if it does, it is for a limited period of time. Future reports and studies are required to confirm our observations.

**Data availability**

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

**Grant information**

PH has research support from Parkinson Canada, Fonds de Recherche Québec – Santé, the Weston Brain Institute, the Natural Sciences and Engineering Research Council of Canada, the Michael J Fox Foundation for Parkinson’s Research and the Montreal Neurological Institute.

**References**


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Current Peer Review Status: ✔️ ✔️

Version 2

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✔️ Cécile Moro
CEA, LETI, CLINATEC, University of Grenoble Alpes, Grenoble, France

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 May 2019
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✔️ Joachim Wistuba
Centre of Reproductive Medicine and Andrology, Institute of Reproductive and Regenerative Biology, Münster, Germany

All my comments were sufficiently dealt with.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Reproductive Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Nuara et al provide a report on marmoset reproduction after MPTP toxin exposure. This case report is interesting as very few is known about it on literature. However, it is more a case report than an experimental plan to study effects of MPTP on reproduction. A chronic exposure to low dose of MPTP would have been interesting to evaluate impact on reproduction, in addition of hormonal balance. Parkinsonian state of animals at conception and during pregnancy would be interesting to know (do they still have symptoms?). In the present study, MPTP was injected for only 5 days, and it is known that MPTP is washed out after a few days in mice, and that brain function recover partly after a few months. I would suggest to adapt discussion to a case report instead of scientific conclusion, and/or indicate that MPTP exposure was temporary.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Macaca fascicularis; parkinsonian model; photobiomodulation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
Author Response 17 May 2019

Philippe Huot, Montreal Neurological Institute and Hospital, Montreal, Canada

However, it is more a case report than an experimental plan to study effects of MPTP on reproduction. This is a concern we have had from the moment we began working on this report, as the manuscript does not precisely fit any of the proposed categories. We went back to the MNI Open Research authors' guidelines, which state that case reports should describe clinical observations. For this reason, acknowledging the caveat mentioned by the Reviewer, we would like to leave the manuscript within the “Research Notes” category.

In the present study, MPTP was injected for only 5 days, and it is known that MPTP is washed out after a few days in mice, and that brain function recover partly after a few months. I would suggest to adapt discussion to a case report instead of scientific conclusion, and/or indicate that MPTP exposure was temporary. See response to the comment above for the first half of the query. We now emphasise that the exposure to MPTP was temporary: “These cases suggest that MPTP, when administered acutely may not be toxic to the reproductive system, in the marmoset”.

Competing Interests: No competing interests were disclosed.

Reviewer Report 13 May 2019

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Joachim Wistuba
Centre of Reproductive Medicine and Andrology, Institute of Reproductive and Regenerative Biology, Münster, Germany

In their report the authors conclude that MPTP, a neuro-toxin used to induce a Parkinson's disease like phenotype in non-human primates has no or only limited effect on reproductive ability in marmoset monkeys. They observed a male marmoset which was able to procreate 1.5 years after toxin administration and a second case of a couple of animals both treated with MPTP producing offspring together.

This report is interesting as it was not yet clear whether or not the toxin interferes with the HPG axis or the local endocrine milieu in primates in a way that might hamper reproductive ability. Moreover the Parkinson-like phenotype appears not to impair brood care behaviour. However, it should be considered that neotropic monkeys have a peculiar endocrine regulation of reproduction and the results seen here might therefore not be fully transferable to other primates.
The report is well designed and gives full insight into the events observed. I have only two questions/comment the authors might consider for discussion:

1. As in case of the pair the female was treated with contraception it was not clear to me why she got pregnant at all? In line with this it might be that MPTP treatment has an effect on the time to pregnancy, which cannot be excluded due to the contraceptive regimen applied to the female. The authors might consider to discuss this point.

2. The endocrine regulation in marmosets is peculiar – thus, it might be that MPTP is not interfering into New World monkeys reproductive regulation, however this does not mean that the same is true in other monkey, incl. the human.

Minor: in Materials and Methods, the authors state that the monkeys had unlimited access to food – is that really true?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Reproductive Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 17 May 2019

Philippe Huot, Montreal Neurological Institute and Hospital, Montreal, Canada

In case of the pair the female was treated with contraception it was not clear to me why she got pregnant at all? In line with this it might be that MPTP treatment has an effect on the time to pregnancy, which cannot be excluded due to the contraceptive regimen applied to the female. The authors might consider to discuss this point.

Indeed, we were equally surprised when it was discovered that the female marmoset became pregnant despite the contraception. The following sentences were added to discuss it: “It is nevertheless surprising that the female marmoset was able to become pregnant despite the monthly administration of contraceptive. It is possible that the injection was not optimally timed...”
within her cycle; whether MPTP slightly interfered with the cycle duration remains uncertain."

*The endocrine regulation in marmosets is peculiar – thus, it might be that MPTP is not interfering into New World monkeys reproductive regulation, however this does not mean that the same is true in other monkey, incl. the human.*

This is an important point about which we did not think while writing the report. We have added this sentence to incorporate it: “It is noteworthy that marmosets are New World monkeys; it is currently unknown whether Old World monkeys, apes or humans would be able to procreate after exposure to MPTP”.

*In Materials and Methods, the authors state that the monkeys had unlimited access to food – is that really true?*

We thank the Reviewer for noticing this detail. We corrected it and it now reads: “They had unlimited access to water and were fed with marmoset food (Mazuri Marmoset Jelly, catalogue Number 0007066, USA), nuts and fresh fruits twice daily”.

**Competing Interests**: No competing interests were disclosed.