Quantitative assessment of motor function in minipig models of neurological disorders using a pressure-sensitive gait mat

Steinmüller, Johannes Bech; Binda, Karina Henrique; Lillethorup, Thea Pinholt; Søgaard, Bjarke; Orlowski, Dariusz; Landau, Anne M.; Bjarkam, Carsten Reidies; Sørensen, Jens Christian Hedemann; Glud, Andreas Nørgaard

Published in:
Journal of Neuroscience Methods

DOI (link to publication from Publisher):
10.1016/j.jneumeth.2022.109678

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Journal of Neuroscience Methods 380 (2022) 109678

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Quantitative assessment of motor function in minipig models of neurological disorders using a pressure-sensitive gait mat

Johannes Bech Steinmüller [a,b,*,1,2], Karina Henrique Binda [c,d,1], Thea Pinholt Lillethorup [c,d], Bjarke Søgaard [a], Dariusz Orlowski [a], Anne M. Landau [c,d], Carsten Reidies Bjarkam [b], Jens Christian Hedemann Sørensen [a], Andreas Nørgaard Glud [a]

[a] CENSE, Department of Neurosurgery, Aarhus University Hospital, and Department of Clinical Medicine, Faculty of Health, Aarhus University, Palle Juul-Jensens Boulevard 165, Entrance J, DK-8200 Aarhus, Denmark
[b] Department of Neurosurgery, Aalborg University Hospital, and Department of Clinical Medicine, Aalborg University, Hobrovej 18-22, DK-9000 Aalborg, Denmark
[c] Department of Nuclear Medicine & PET-Center, Aarhus University, Palle Juul-Jensens Boulevard 165, Entrance J, DK-8200 Aarhus, Denmark
[d] Translational Neuropsychiatry Unit, Aarhus University, Universitetsbyen 13, 2B, DK-8000 Aarhus, Denmark

1. Background

Neuroscientific research using minipigs is a rapidly evolving field. Indeed, the minipig has been used as a translational large, non-primate animal model of several neurological diseases including Parkinson's disease (PD) (Bjarkam et al., 2008; Glud et al., 2011; Lillethorup et al., 2018a, 2018b; Mikkelsen et al., 1999; Nielsen et al., 2016), Huntington's disease (Ardan et al., 2019; Schramke et al., 2016), ischemic stroke (Duberstein et al., 2014), intracerebral hemorrhage (Yang et al., 2020), gliomas (Khoshnevis et al., 2017, 2020; Tora et al., 2020), and spinal cord injury (Jutzeler et al., 2019). Many of these pathologies have a considerable impact on the motor system resulting in motor function deterioration. Previously, many different aspects of the central nervous system function and pathology have been explored in rodent models. Such yield both feasible and considerably cheaper experimental setups as compared to those involving non-human primates. Still, although rodents have a high translational quality, they possess obvious neuroanatomical and physiological differences to humans, e.g., in the sense of their smaller lissencephalic brain and body size (Gieling et al., 2011; Lind et al., 2007; Sorensen et al., 2011). Large animal models hence constitute valuable stepping stones in translating small animal basic research to clinical implementation. Due to their human resembling brain, non-human primates have been used in various aspects of neuroscience, but such models suffer from ethical concerns and substantial research expenditure (Goodman and Check, 2002). This has necessitated alternative candidates.

During the past decade the minipig neuroanatomy has been increasingly characterized (Bech et al., 2020; Bjarkam et al., 2017; Ettrup et al., 2010; Larsen et al., 2004; Meidahl et al., 2016; Nielsen et al., 2009; Tvilling et al., 2021) including different aspects of the motor system (Bech et al., 2018; Larsen et al., 2004; Nielsen et al., 2009; Steinmüller et al., 2021). Several neurological diseases manifest as motor symptoms resulting from dysfunction of the pyramidal or extrapyramidal system. Accordingly, the degree of motor affection acts as a surrogate for pathology progression. Such motor deficits can be quantified with neurological testing thus providing a valuable tool in both acute and chronic animal models. It is crucial, however, that assessments are reproducible, consistent, and unbiased to reliably reflect and correlate with the underlying pathology. Clinically derived scoring systems have been proposed for grading motor symptoms in minipig PD models through structured examination of different motor qualities (Mikkelsen et al., 1999; Moon et al., 2014). However, as neurological examination in general, these approaches are investigator-dependent and bias susceptible, and it may be challenging to achieve rigid scoring over time in chronic models.

Recent years have seen the introduction of quantitative methods using external apparatuses to measure the motor function of minipigs through gait analysis (Glud et al., 2010; Netzley et al., 2021; Schramke et al., 2016; Seo et al., 2020; Thorup et al., 2008, 2007), which has been applied to quantify motor deterioration following ischemic stroke (Duberstein et al., 2014). Many of these analyses are based on video recordings of animals with attached sensors or markers. This requires controlled and relatively extensive experimental setups. However, simple, and easy-to-use devices also exist. Among these is the GAITRite®

* Corresponding author at: CENSE, Department of Neurosurgery, Aarhus University Hospital, and Department of Clinical Medicine, Faculty of Health, Aarhus University, Palle Juul-Jensens Boulevard 165, Entrance J, DK-8200 Aarhus N, Denmark.
E-mail address: jb@clin.au.dk (J.B. Steinmüller).
[1] Shared first authors
[2] ORCID iD: 0000-0002-3833-9566

https://doi.org/10.1016/j.jneumeth.2022.109678
Received 11 May 2022; Received in revised form 7 July 2022; Accepted 20 July 2022
Available online 22 July 2022

pressure sensitive gait mat that has been shown to correlate with the clinical Unified Parkinson's Disease Rating Scale (UPDRS) in the L-DOPA derived improvement seen in PD patients (Menz et al., 2004). Furthermore, the reliability of this gait mat has been supported in a subsequent systematic review (Godinho et al., 2016).

This methodological study aims to provide a quantitative and simple framework for gait analysis of minipigs. This will provide an accessible work tool for the assessment of compromised motor function in various translational models of neurological disorders. The inclusion of video material will assist in demonstrating the setup to increase both the comprehension and validity of the concept.

2. Materials and methods

2.1. Animals

In this study we used 7 female Göttingen minipigs aged 7–10 months and weighing 19.2–26.5 kg. The study was approved by the Danish National Council of Animal Research Ethics (Protocol no.: 2020–15–0201–00710). Animals were kept in pairs or neighboring pens permitting physical contact in approved, enriched housing facilities. Animals had ad libitum access to water, were on controlled diet and weight control, and received continuous care by professional veterinarian staff.

2.2. Training

All animals were trained for 3 weeks prior to gait testing. The training consisted of classic Pavlovian conditioning using a custom-made "clicker stick". Animals were rewarded with a treat (e.g., a piece of apple) when touching the soft tip of the device with the snout following a click. Even after minimal training, the animals could be directed to walk steadily and uniformly along an investigator-instructed direction or along a gait mat (see later Video 1 and Video 2).

2.3. GAIT4Dog® pressure-sensitive gait mat

We used the GAIT4Dog® (CIR Systems Inc., NJ, US) GAITFour® gait mat and accompanying software (Version 4.9Wr) for the quadruped gait analysis. The mat dimension was 5.7 × 0.8 m and was covered with a thin carpet to protect sensors from direct contact or contamination from animals. Pressure-sensitive sensors recorded at a scan rate of 120 Hz. The gait mat was aligned alongside the outer walls of empty pens restricting movement off the mat to this side. One investigator walked on the opposite side to guide the minipigs to walk straight along the mat. The collected data contained: 1) temporal gait data of animal velocity (cm/sec), stance time (ground contact of each limb per gait cycle), stance % (percentage of gait cycle with limb ground contact), 2) spatial gait data of step length (distance between current limb contact and previous limb contact) and stride length (distance between current limb contact and previous contact of the same limb), and 3) pressure data of total pressure index (percent of weight distribution to each limb). To differentiate misleading normal variation between successive gait tests from the general motor assessment, the included data were normalized by calculating the mean of three separate successful gaits. Data were collected from seven healthy minipigs to determine normal reference intervals of a walking gait style (i.e., not running or galloping). All gait testing was performed by the same investigators to yield as uniform data acquisition as possible.

2.4. Surgery and pathological gait analysis

To provide an example of pathological gait dynamics in a translational minipig model, we repeated the gait analyses of the seven minipigs 2–7 weeks after inducing a lesion (n = 5) or a sham lesion (n = 2) serving as control. Animals underwent neurosurgery with unilateral stereotaxic microinjections of either 6-hydroxydopamine (6-OHDA) or saline in the right medial forebrain bundle, as part of a different study. This neurotoxin selectively targets and induces acute cell death of the nigrostriatal catecholaminergic neurons (Ungerstedt, 1968). The resulting loss of dopamine delivered to the basal ganglia from dopaminergic terminals has been shown already one week after injection (Molinet-Dronda et al., 2015) and yields a hemi-parkinsonian phenotype in the lesioned animals. The dopaminergic depletion was assessed with positron emission tomography (PET) as part of a parallel study.

Animals were sedated with an intramuscular injection of 4 mL ketamine (25 mg/mL, Pfizer®) and 6 mL midazolam (5 mg/mL, Hameln®) prior to ear vein cannulation. An additional sedative dose was intravenously administered, and minipigs were intubated for continuous 2 % sevoflurane general anaesthesia as previously described (Ettrup et al., 2011). Next, the minipigs received a bladder catheter and were fixated in an MRI-compatible head frame used for the stereotaxic procedure (Bjarkam et al., 2004; Ettrup et al., 2011). Animals received buprenorphine analgesics and antibiotics (Cefuroxim "Fresenius Kabi" 1500 mg), and infiltrative analgesia with 10 mL bupivacaine (Marcaine® adrenalin 5 mg/mL + 5 μg/mL, Aspen Pharmacare) was subcutaneously placed at the zygomatic screws and skull midline. A midline incision was made, and a fiducial marker inserted in a skull burr hole (Glud et al., 2017). Anatomical MRIs were made (Siemens 3 T Magnetom Skyra, 3D T1-weighted sequence, slice thickness 1 mm, voxel size 1×1x1 mm^3, 176 slices, FOV = 256 mm, TR = 2420 ms, TE = 3.7 ms, 2 averages, TI = 960 ms, and flip angle = 9 degrees). Stereotaxic coordinates were defined by measuring distances from the fiducial marker in the accompanying MRI software (Glud et al., 2017). A craniotomy was made over the cortical entry point of the stereotaxic target trajectory and the dura was carefully incised with a dura knife. Using a Hamilton microsyringe, three injections of 25 μL 6-OHDA (10 mg/mL, Sigma-Aldrich®) or saline were placed 1 mm apart in the right medial forebrain bundle between the substantia nigra and subthalamic nucleus as has previously been done (Christensen et al., 2018). We used an infusion rate of 5 μL/min before careful, stepwise retraction to prevent backflow along the needle track. Finally, the scalp was sutured.

2.5. Statistics

All statistical analyses were conducted in GraphPad Prism (Version 9.2.0, GraphPad Software, LLC, San Diego, CA, US). We performed descriptive statistics of animal characteristics and basic gait parameters to suggest a reference interval of healthy minipigs according to age and weight. Continuous data were presented as medians with interquartile range [IQR] or as means with standard deviation (SD) for parametric data. Step length, stride length, stance time, stance %, and total pressure index of extremities were considered non-parametric data. Significant variation of each gait parameter, except total pressure index, was analyzed using a Kruskal-Wallis test, both across individual extremities and across animals. Velocity, stance time, and step length data were tested for normality using a QQ plot (velocity data) or the Anderson-Darling test (stance time and step length). Accordingly, statistical significance differences of normally distributed data (velocity and step length) were assessed using paired t-tests. We chose a Wilcoxon matched-pairs signed-rank test to compare stance times since these data were not normally distributed. For step length and stance time, we compared the respective right and left extremities (ipsilateral or contralateral to lesions) to assess possible effects of our hemi-parkinsonian model. We did not perform statistical comparisons of the saline controls as this sample size consisted of only 2 animals.

3. Results

3.1. Animals

The seven female minipigs had a median age of 8 months IQR= [8–9]

Table 1
Animal characteristics.

Animal no.	Sex	Age *months*	Weight *kg*
Minipig 1	Female	9	25.9
Minipig 2	Female	8	26.5
Minipig 3	Female	10	26.4
Minipig 4	Female	8	19.5
Minipig 5	Female	7	19.5
Minipig 6	Female	8	19.2
Minipig 7	Female	8	19.5
Median [IQR]		8 [8–9]	19.5 [19.5–26.4]

Sex, age, and weight data of animals. Medians with interquartile range are shown in the bottom row.

and a median weight of 19.5 kg IQR= [19.5–26.4] (Table 1). All animals could be trained to walk in a controlled manner on the gait mat. Some animals were initially very eager to perform the gait testing, which resulted in poorly controlled tests of varying velocity and direction. After several walks, the animals were more prone to instructions and performed more straight and steady walks. This was important to account for when acquiring the gait data to increase the consistency over time and the robustness of the data.

3.2. Gait analyses

We first analyzed the gait data obtained at baseline to provide healthy reference values and gait characteristics. We found a symmetrical, similar step length and stride length, respectively, across the four extremities within individual animals. No statistically significant variation was found for these parameters, although a greater variance between extremities was seen for the stance % of the hind limbs. However, animals were found to apply more pressure on the front limbs that hence supported most of their body weight during walking. This results in a center of gravity that is anteriorly located compared to that of humans. This pressure difference was supported by significant statistical variation ($P = 0.0001$). Data are shown in Table 2 and summarized in Fig. 1. When assessing data across animals, we found that animals significantly varied when analyzing all step lengths ($P = 0.0003$), stride lengths ($P = 0.0002$), stance times ($P = 0.0002$), and stance % ($P = 0.0310$).

Second, we compared the baseline data with the post-surgical data from either the 6-OHDA lesioned or sham lesioned animals to shed light on the pathological gait dynamics of our applied model (see Table 3 and Fig. 2). The baseline mean velocity of the 6-OHDA lesioned minipigs was 102.4 cm/sec ($SD = 8.533$) and ranged from 90.8 cm/sec to 114.3 cm/sec. This was despite the exclusion of walks where animals were running or galloping. After surgically causing the lesion to induce a parkinsonian phenotype, the mean velocity significantly decreased to 90.42 cm/sec ($SD = 11.82$) ($P = 0.0275$). As for the controls, the baseline mean velocity was 102.7 cm/sec ($SD = 1.393$), which was largely unaltered at 101.2 cm/sec ($SD = 6.081$) after sham lesions. For the remaining gait parameters, we segmented the analysis into the right limbs (ipsilateral to the lesion) and left limbs (contralateral to the lesion).

At baseline, the median stance times were 0.295 s [0.258–0.343] in the right limbs and 0.285 s [0.26–0.353] in the left limbs of the 6-OHDA lesioned animals. These significantly increased to 0.32 s [0.298–0.418]

Table 2
Normal gait characteristics.

	Step Length				Stride Length				Stance Time				Stance %				Total Pressure Index			
	cm				cm				sec				%				%			
Animal no.	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*
Minipig 1	27.77	28.63	28.03	28.10	56.27	56.57	56.00	56.20	0.33	0.32	0.34	0.33	59.73	57.50	59.00	59.60	31.17	26.13	21.77	20.90
Minipig 2	26.60	26.87	25.90	27.33	53.43	53.30	53.30	53.30	0.31	0.32	0.32	0.32	58.13	59.40	58.03	58.77	29.00	29.30	20.43	21.40
Minipig 3	28.80	29.37	29.80	28.33	58.23	58.10	58.17	58.03	0.40	0.38	0.39	0.39	60.23	59.53	59.43	58.13	27.97	29.17	21.53	21.17
Minipig 4	24.30	26.60	25.23	25.73	51.13	51.17	51.03	51.13	0.31	0.29	0.30	0.30	60.10	57.73	57.40	58.23	28.40	25.30	23.23	22.70
Minipig 5	24.23	25.13	24.70	24.83	49.50	49.43	49.70	49.47	0.28	0.29	0.29	0.30	56.77	58.57	57.07	59.90	28.93	31.27	18.13	21.13
Minipig 6	23.47	23.63	23.93	23.30	47.23	47.03	47.30	47.23	0.28	0.26	0.26	0.26	58.67	54.63	54.90	56.43	32.10	30.37	18.73	18.63
Minipig 7	25.13	25.63	25.70	25.10	50.77	50.87	50.77	51.00	0.26	0.25	0.21	0.21	57.80	55.27	46.37	48.10	30.13	28.5	20.97	20.57
Median	25.13	26.60	25.70	25.73	51.13	51.17	51.03	51.13	0.31	0.29	0.30	0.30	58.67	57.73	57.40	58.23	29.00	29.17	20.97	21.13
IQR	[24.23-27.77]	[25.13-28.63]	[24.70-28.03]	[24.83-28.10]	[49.50-56.27]	[49.43-56.57]	[49.70-56.00]	[49.47-56.20]	[0.28-0.33]	[0.26-0.32]	[0.26-0.34]	[0.26-0.33]	[57.80-60.10]	[55.27-59.40]	[54.90-59.00]	[56.43-59.60]	[28.40-31.17]	[26.13-30.37]	[18.73-21.77]	[20.57-21.40]

A summary of normalized baseline data, i.e., all values represent means calculated from data of three separate walks generated in the GAITFour® software. Medians and interquartile ranges of the individual limbs are listed below. LF = left front limb, RF = right front limb, LH = left hind limb, RH = right hind limb.

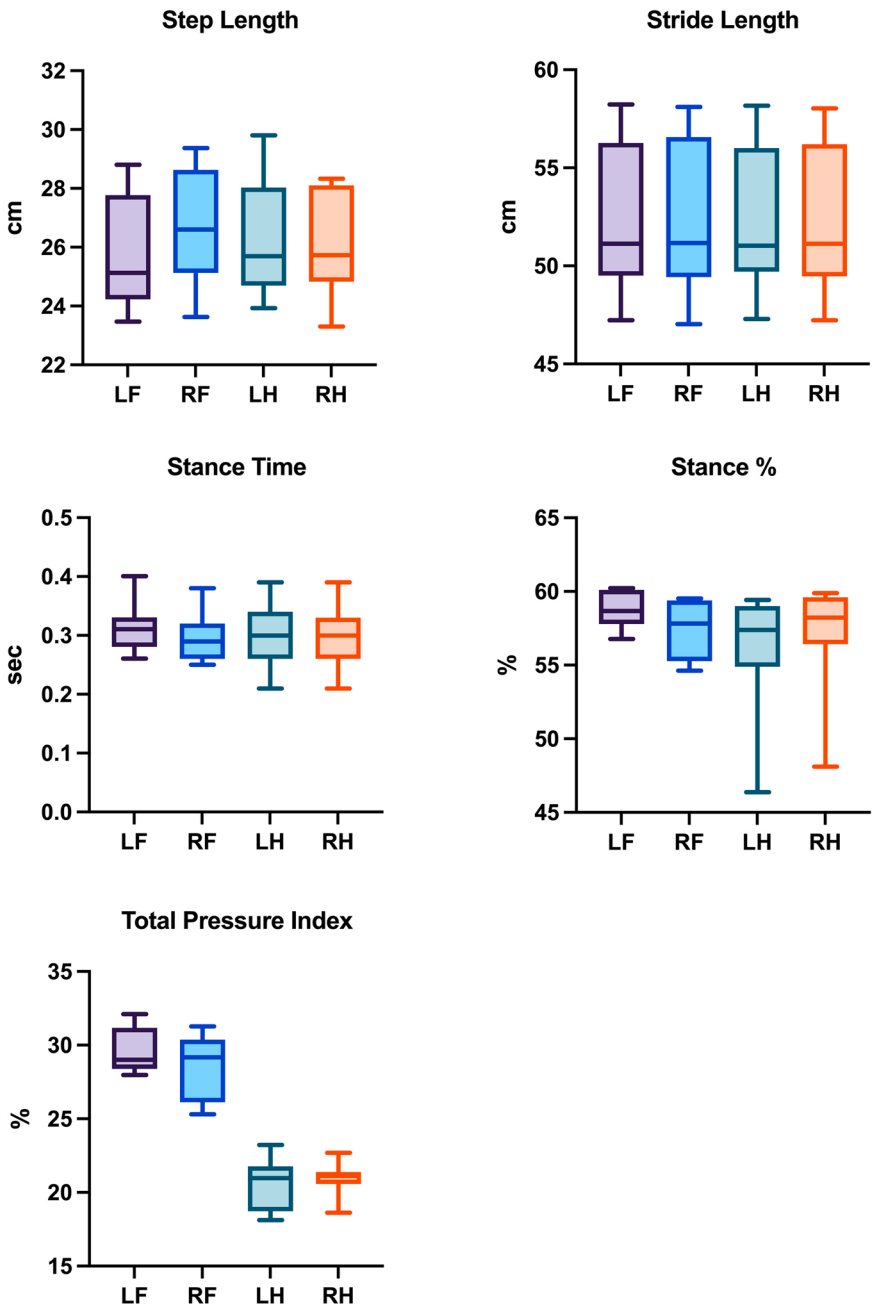

Fig. 1. Normal gait parameters.
Box and whisker plots depicting the median, interquartile range, and maximum range of normal gait parameter values for each limb. Stance % is the percentage of the gait cycle, where the animals have ground-contact. Total pressure index is the percentage of pressure provided by each individual limb. See also text for details. A significant variation was found for total pressure index (P = 0.0001), but no significant variation was found for the remaining parameters across extremities. LF = left front limb, RF = right front limb, LH = left hind limb, RH = right hind limb.

in the right limbs ($P = 0.0059$) and 0.325 s [0.298–0.405] in the left limbs ($P = 0.0117$) after lesions. The controls had a baseline median stance time of 0.31 s [0.293–0.32] in the right limbs and 0.31 s [0.303–0.318] in the left limbs, which slightly decreased to 0.295 s [0.29–0.308] in the right limbs ($P = 0.25$) while being unaltered in the left limbs.

The step lengths of the right limbs of 6-OHDA animals at baseline had a mean of 26.21 cm (SD = 2.203), whereas the left limbs had a mean step length of 26.16 cm (SD = 2.252). For the ipsilateral right limbs this mean step length significantly decreased to 25.53 cm (SD = 2.339) ($P = 0.0083$) in the 6-OHDA lesioned animals, but increased in the contralateral left limbs to 26.75 (SD = 1.387), although not significantly ($P = 0.2857$). The saline animals had baseline mean step lengths of 26.63 cm (SD = 0.673) in the right limbs and 25.51 cm (SD = 0.98) in the left limbs, which were slightly increased after sham lesions to 26.74 cm (SD = 0.415) in the right limbs and 25.96 cm (SD = 1.245) in the left limbs.

3.3. Gait videos

To demonstrate the gait testing, we made two video sequences of gait data acquisition. Especially since an obvious pitfall and concern of the method is that the investigator is determining the velocity, which would introduce bias in the data. We, therefore, provide video material as documentation. Video 1 is showing a saline sham-lesioned animal performing a normal gait in three sequences: 1) normal, regular walk as during data acquisition. Note how the investigator instructs the animal to follow the "click stick", 2) a faster pace as instructed by the investigator, and 3) a very fast walk where the instructor forces the animal to increase its velocity markedly. These repeated sequences display that in healthy minipigs, the investigator can potentially affect the velocity if care is not taken to be consistent, or if investigators change. Video 2 is showing a 6-OHDA lesioned animal, where the investigator: 1) instructs the animal during data acquisition, 2) attempts to increase the velocity of the animal slightly, and 3) tries to maximally increase the velocity.

Table 3
Velocity, stance time, and step length dynamics.

		Surgery	Velocity cm/sec		Stance Time sec		Step Length cm	
Animal no.	**Limb**	*Lesion/sham*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Minipig 1	LF	6-OHDA	104.67	99.73	0.33	0.33	27.77	26.93
	RF				0.32	0.33	28.63	29.20
	LH				0.34	0.31	28.03	28.17
	RH				0.33	0.31	28.10	27.93
Minipig 2	LF	Saline	101.73	105.47	0.31	0.32	26.60	26.93
	RF				0.32	0.31	26.87	27.10
	LH				0.32	0.31	25.90	27.13
	RH				0.32	0.30	27.33	27.07
Minipig 3	LF	6-OHDA	90.80	75.60	0.40	0.56	28.80	27.63
	RF				0.38	0.50	29.37	28.33
	LH				0.39	0.51	29.80	29.07
	RH				0.39	0.54	28.33	26.73
Minipig 4	LF	Saline	103.70	96.87	0.31	0.31	24.30	24.77
	RF				0.29	0.29	26.60	26.50
	LH				0.30	0.30	25.23	25.00
	RH				0.30	0.29	25.73	26.27
Minipig 5	LF	6-OHDA	102.83	79.93	0.28	0.37	24.23	24.70
	RF				0.29	0.39	25.13	24.87
	LH				0.29	0.37	24.70	26.17
	RH				0.30	0.36	24.83	23.47
Minipig 6	LF	6-OHDA	99.40	95.73	0.28	0.32	23.47	26.77
	RF				0.26	0.30	23.63	23.20
	LH				0.26	0.29	23.93	27.40
	RH				0.26	0.30	23.30	22.67
Minipig 7	LF	6-OHDA	114.33	101.10	0.26	0.30	25.13	25.17
	RF				0.25	0.29	25.63	24.70
	LH				0.21	0.26	25.70	25.47
	RH				0.21	0.26	25.10	24.20
Mean or Median(†)	R	6-OHDA	102.40	90.42	0.295†	0.320†	26.21	25.53
	L				0.285†	0.325†	26.16	26.75
	R	Saline	102.7	101.2	0.310†	0.295†	26.63	26.74
	L				0.310†	0.310†	25.51	25.96
(SD) or [IQR]	R	6-OHDA	(8.533)	(11.82)	[0.258–0.343]	[0.298–0.418]	(2.203)	(2.339)
	L				[0.260–0.353]	[0.298–0.405]	(2.252)	(1.387)
	R	Saline	(1.393)	(6.081)	[0.293–0.320]	[0.290–0.308]	(0.673)	(0.415)
	L				[0.303–0.318]	[0.303–0.318]	(0.980)	(1.245)

Pre/post-surgical gait data from all minipigs receiving either a lesion (6-OHDA) or sham lesion (saline). Investigated parameters included velocity, stance time, and step length. The bottom two rows are the estimated mean velocity (SD), median stance time [IQR], and mean step length (SD). A significant decrease in velocity ($P = 0.0275$) and increase in stance time (right $P = 0.0059$; left $P = 0.0117$) was seen for 6-OHDA animals. A significant decrease in step length was seen only in the right limbs of the 6-OHDA animals ($P = 0.0083$) ipsilateral to the induced lesions. No significant changes were seen in the saline controls. See also Fig. 2 for an overview. LF = left front limb, RF = right front limb, LH = left hind limb, RH = right hind limb, R = right, L = left. † = Median.

Here it is evident that the animal is unable to increase the velocity due to the induced lesion. Also note the subtle bradykinesia, where an increased stance time can be appreciated. These videos demonstrate two important considerations; first that in healthy animals, care must be taken to obtain consistency of the gait data and, second, that lesioned animals with pathological gait may not be able to compensate.

Supplementary material related to this article can be found online at doi:10.1016/j.jneumeth.2022.109678.

4. Discussion

The data presented in this study provides reference values of several gait parameters in healthy, adult minipigs. Additionally, the video material provides an intelligible demonstration of the experimental setup simplicity and how to acquire the data. When comparing healthy baseline gait parameter values with those obtained in hemiparkinsonian minipigs after 6-OHDA lesion or sham, only the lesioned animals were seen to have a decrease in velocity, increase in stance time and decrease in step length, whereas the sham animals were unaffected.

4.1. Normal gait characteristics

The minipigs used in this study ranged from 7 to 10 months of age. A previous study has determined the sexual maturation age of female Göttingen minipigs to 3.7–6.5 months (Peter et al., 2016) and, accordingly, we argue that our proposed gait references represent those of mature and adult animals. A recent study reported behavioral changes in locomotion in an open-field test indicating that adult minipigs were more courageous in exploring their environment (Netzley et al., 2021). It is possible that changes in gait parameters also occur alongside maturation. This would be difficult to differentiate from the effects of repeated training or testing. The finding that only slight changes occurred in the control animals from baseline until the testing after sham lesions opposes this possibility, although being based on merely two animals. Still, our investigated animals were young adults, and it might be worth considering age as a factor for future experiments. Besides age, the sex of animals could potentially influence gait characteristics. Female minipigs may be preferable to males since they are less aggressive, docile, and easy to work with. Therefore, all animals used in our study were females. Providing reference gait characteristics including those of male minipigs might provide more nuanced characteristics. Contrarily, a previous study found no significant differences between sexes on gait parameters (Thorup et al., 2007). Our reported parameter values indicated gait symmetry which has also been reported by others (Duberstein et al., 2014). The finding that animals applied more pressure on the front limbs is also in agreement with previous studies (Thorup et al., 2008,

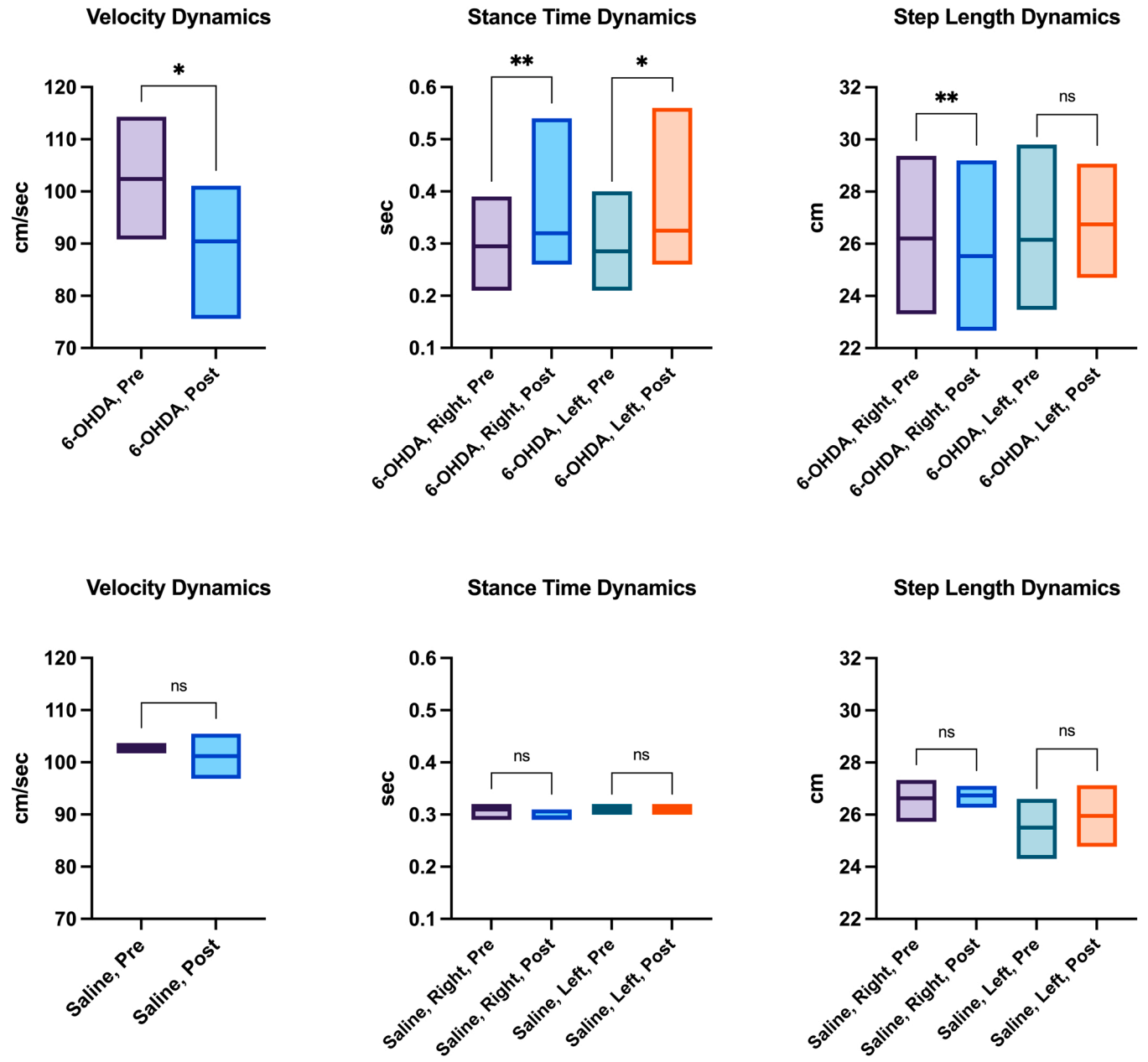

Fig. 2. Gait dynamics after surgery.
Box plots with means (or medians in the stance time bars) and maximum range depicting the animal gait dynamics categorized into velocity, stance time and step length. Lesioned animals (n = 5) with 6-OHDA neurotoxin (top row) and saline controls (n = 2) (bottom row) are plotted. A significant decrease was found for the velocity post-surgery in the 6-OHDA lesioned animals (P = 0.0275). The stance times were significantly increased for both the right (P = 0.0059) and left (P = 0.0117) limbs of the lesioned animals. The step length only significantly decreased in the ipsilateral right limbs of 6-OHDA lesioned animals (P = 0.0083). No significant dynamic changes were observed in either gait parameter in the saline controls. ns = not significant.

2007) and indicated a slightly frontal center of gravity.

4.2. Lesion models

Previously, different motor aspects including muscle rigidity, mobility, and positional abnormity have been used for longitudinal evaluation of motor function (Mikkelsen et al., 1999). These neurological assessments have been implemented in the daily care and observation of our animals and are regularly registered by the veterinary staff. We assessed this record data from Minipig 1–7, and no such abnormal changes were registered by the veterinary staff before or after the animals received nigrostriatal lesions. The only pathological observation was a temporarily reduced chewing function in one of the 6-OHDA lesioned minipigs (minipig no. 3) that remitted spontaneously. As can be appreciated in Table 3, this animal had the lowest velocity and longest stance time, and was clinically most affected. In fact, this animal also displayed the most pronounced spontaneous rotational behavior, as has previously been reported post-surgery in this lesion model (Christensen et al., 2018). To a lesser degree, this behavior was also observed in minipig no. 6. While the remaining lesioned animals had more discrete symptoms, this exemplifies the need for quantitative approaches to detect subtle motor deviations. Importantly, we found that our gait analysis detected such minor changes, although the dynamics generally correlated with the clinically observed motor deterioration. The reported changes of gait parameters likely represent a combination of direct motor involvement resulting from a compromised

extrapyramidal system and compensatory mechanisms to maintain postural balance. Such was indicated by the significant decrease in velocity after the induced 6-OHDA lesions. This is consistent with another study reporting reduced walking speed compensating for gait insecurity on slippery floor conditions (Thorup et al., 2007). Furthermore, we found significant compensative increases in stance time, which has similarly been reported (Thorup et al., 2007). Our results revealed dynamic alterations in step length, but only in the ipsilateral right limbs. Although such a decreased step length is well-known in PD, we expected that changes would be most pronounced on the side contralateral to the PD lesion. A recent human study found contralaterally decreased rigidity and bradykinesia after unilateral pallidothalamic tractotomy treatment of akinetic-rigid PD patients (Horisawa et al., 2021). However, the mechanisms behind gait control are complex and it has previously been shown that the pyramidal tract of minipigs contains 7–19 % uncrossed fibers (Bech et al., 2018). In humans, uncrossed pyramidal fibers are important for posture and truncal stability, which can hypothetically constitute an anatomical rationale for the observed ipsilateral gait symptomatology.

Significant variation across animals was found when investigating gait parameters. This emphasizes that the motor impact of any induced lesion must surpass this normal variation to be detectable. Indeed, it is difficult to recognize that the seemingly small gait parameter changes translate into clinically significant symptomatology. We argue that other models with more prominent motor dysfunction would likely produce more striking differences in the gait data since at least some correlation between the observable and measurable deficits is rational. This suggestion is supported by a previous study using a stroke model, where the stance and hoof height were affected, although to a varying degree (Duberstein et al., 2014). In this study, however, only two animals were lesioned with a middle cerebral artery occlusion. Even though such a lesion may considerably affect the motor system, an anatomical description of the ischemic lesion including the involved cortical areas or white matter pathways was not made. This introduces some uncertainty in predicting the expected gait disturbances. Contrarily, applying models with severe motor impact may also bring about difficulties as described in another study (Moon et al., 2014), where only one of three parkinsonian MPTP-lesioned animals survived for full analysis. This underlines the importance of choosing a balanced and carefully selected lesion model when conducting translational studies involving motor deficits in minipigs.

4.3. GAITFour® methodological considerations

Another important consideration when using the gait mat method is the importance of using the same investigators to instruct the animals in order to secure uniform and comparable results. This is important since some parameters, including velocity, may be subject to investigator-derived variation in healthy animals, which could potentially interfere with baseline testing and thus obscure gait dynamics found after subsequent post-lesion testing. It is also important to consider how treats are used to instruct animals. Emphasis must be placed on uniformly conducting the tests and critically assessing the steadiness of the performed walks. We deem a lack of stringency could potentially introduce bias in the data.

5. Conclusion

With this study we have provided a simple framework for acquiring quantitative gait data of minipigs using a pressure-sensitive gait mat. Analysis of such data may have important value in translational minipig models implementing motor dysfunction. With our results we have provided references to be used for future studies and demonstrated that the method is sensitive enough to detect and quantify subtle motor deterioration that was otherwise not detected by mere clinical assessment. Still, the detectable changes in locomotion are likely correlated with the degree of motor dysfunction. Therefore, we recommend that both the selection and degree of applied lesions deserve careful consideration in minipig models of neurologic diseases to balance gait data quality and animal welfare.

CRediT authorship contribution statement

Johannes Bech Steinmüller: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Karina Henrique Binda:** Investigation, Writing – review & editing, Visualization. **Thea Pinholt Lillethorup:** Methodology, Validation, Supervision, Writing – review & editing. **Bjarke Søgaard:** Visualization. **Dariusz Orlowski:** Supervision, Writing – review & editing. **Anne M. Landau:** Supervision, Funding acquisition, Writing – review & editing. **Carsten Reidies Bjarkam:** Supervision, Funding acquisition, Writing – review & editing. **Jens Christian Hedemann Sørensen:** Conceptualization, Funding acquisition, Project administration. **Andreas Nørgaard Glud:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration.

Conflict of Interest

The authors declare no conflict of interests.

Acknowledgements

We gratefully acknowledge the received funding from the Independent Research Fund Denmark (No. 0134-00226B), the Jascha Foundation (No. 2021-0110), “Søster og Verner Lipperts Fond”, “Bjarne Saxhofs Fond” administered through the “Parkinsonforeningen”, and “Frimodt-Heineke Fonden”. We thank Aarhus University for providing the salary of KHB. Last, we are thankful for the invaluable assistance of Lise Moberg Fitting, Anne Sofie Møller Andersen, Aage Kristian Olsen Alstrup, and the veterinary staff at Påskehøjgård.

References

Ardan, T., Baxa, M., Levinská, B., Sedláčková, M., Nguyen, T.D., Klíma, J., Juhás, Š., Juhásová, J., Šmatlíková, P., Vochozková, P., Motlík, J., Ellederová, Z., 2019. Transgenic minipig model of Huntington’s disease exhibiting gradually progressing neurodegeneration. Dis. Model Mech. 13.

Bech, J., Glud, A.N., Sangill, R., Petersen, M., Frandsen, J., Orlowski, D., West, M.J., Pedersen, M., Sørensen, J.C.H., Dyrby, T.B., Bjarkam, C.R., 2018. The porcine corticospinal decussation: a combined neuronal tracing and tractography study. Brain Res. Bull. 142, 253–262.

Bech, J., Orlowski, D., Glud, A.N., Dyrby, T.B., Sørensen, J.C.H., Bjarkam, C.R., 2020. Ex vivo diffusion-weighted MRI tractography of the Göttingen minipig limbic system. Brain Struct. Funct. 225, 1055–1071.

Bjarkam, C.R., Cancian, G., Larsen, M., Rosendahl, F., Ettrup, K.S., Zeidler, D., Blankholm, A.D., Ostergaard, L., Sunde, N., Sorensen, J.C., 2004. A MRI-compatible stereotaxic localizer box enables high-precision stereotaxic procedures in pigs. J. Neurosci. Methods 139, 293–298.

Bjarkam, C.R., Glud, A.N., Orlowski, D., Sorensen, J.C., Palomero-Gallagher, N., 2017. The telencephalon of the Gottingen minipig, cytoarchitecture and cortical surface anatomy. Brain Struct. Funct. 2093–2114.

Bjarkam, C.R., Nielsen, M.S., Glud, A.N., Rosendal, F., Mogensen, P., Bender, D., Doudet, D., Moller, A., Sorensen, J.C., 2008. Neuromodulation in a minipig MPTP model of Parkinson disease. Br. J. Neurosurg. 22 (Suppl 1), S9–S12.

Christensen, A.B., Sorensen, J.C.H., Ettrup, K.S., Orlowski, D., Bjarkam, C.R., 2018. Pirouetting pigs: a large non-primate animal model based on unilateral 6-hydroxydopamine lesioning of the nigrostriatal pathway. Brain Res. Bull. 139, 167–173.

Duberstein, K.J., Platt, S.R., Holmes, S.P., Dove, C.R., Howerth, E.W., Kent, M., Stice, S. L., Hill, W.D., Hess, D.C., West, F.D., 2014. Gait analysis in a pre- and post-ischemic stroke biomedical pig model. Physiol. Behav. 125, 8–16.

Ettrup, K.S., Glud, A.N., Orlowski, D., Fitting, L.M., Meier, K., Soerensen, J.C., Bjarkam, C.R., Alstrup, A.K., 2011. Basic surgical techniques in the Gottingen minipig: intubation, bladder catheterization, femoral vessel catheterization, and transcardial perfusion. J. Vis. Exp.: JoVE 52, 2652.

Ettrup, K.S., Sørensen, J.C., Bjarkam, C.R., 2010. The anatomy of the Göttingen minipig hypothalamus. J. Chem. Neuroanat. 39, 151–165.

Gieling, E.T., Schuurman, T., Nordquist, R.E., van der Staay, F.J., 2011. The pig as a model animal for studying cognition and neurobehavioral disorders. Curr. Top. Behav. Neurosci. 7, 359–383.

Glud, A.N., Bech, J., Tvilling, L., Zaer, H., Orlowski, D., Fitting, L.M., Ziedler, D., Geneser, M., Sangill, R., Alstrup, A.K.O., Bjarkam, C.R., Sorensen, J.C.H., 2017.

A fiducial skull marker for precise MRI-based stereotaxic surgery in large animal models. J. Neurosci. Methods 285, 45–48.
Glud, A.N., Hedegaard, C., Nielsen, M.S., Sorensen, J.C., Bendixen, C., Jensen, P.H., Larsen, K., Bjarkam, C.R., 2010. Direct gene transfer in the Gottingen minipig CNS using stereotaxic lentiviral microinjections. Acta Neurobiol. Exp. 70, 308–315.
Glud, A.N., Hedegaard, C., Nielsen, M.S., Sørensen, J.C., Bendixen, C., Jensen, P.H., Mogensen, P.H., Larsen, K., Bjarkam, C.R., 2011. Direct MRI-guided stereotaxic viral mediated gene transfer of alpha-synuclein in the Göttingen minipig CNS. Acta Neurobiol. Exp. 71, 508–518.
Godinho, C., Domingos, J., Cunha, G., Santos, A.T., Fernandes, R.M., Abreu, D., Gonçalves, N., Matthews, H., Isaacs, T., Duffen, J., Al-Jawad, A., Larsen, F., Serrano, A., Weber, P., Thoms, A., Sollinger, S., Graessner, H., Maetzler, W., Ferreira, J.J., 2016. A systematic review of the characteristics and validity of monitoring technologies to assess Parkinson's disease. J. Neuroeng. Rehabil. 13, 24.
Goodman, S., Check, E., 2002. The great primate debate. Nature 417, 684–687.
Horisawa, S., Fukui, A., Yamahata, H., Tanaka, Y., Kuwano, A., Momosaki, O., Iijima, M., Nanke, M., Kawamata, T., Taira, T., 2021. Unilateral pallidothalamic tractotomy for akinetic-rigid Parkinson's disease: a prospective open-label study. J. Neurosurg. 1–7.
Jutzeler, C.R., Streijger, F., Aguilar, J., Shortt, K., Manouchehri, N., Okon, E., Hupp, M., Curt, A., Kwon, B.K., Kramer, J.L.K., 2019. Sensorimotor plasticity after spinal cord injury: a longitudinal and translational study. Ann. Clin. Transl. Neurol. 6, 68–82.
Khoshnevis, M., Carozzo, C., Bonnefont-Rebeix, C., Belluco, S., Leveneur, O., Chuzel, T., Pillet-Michelland, E., Dreyfus, M., Roger, T., Berger, F., Ponce, F., 2017. Development of induced glioblastoma by implantation of a human xenograft in Yucatan minipig as a large animal model. J. Neurosci. Methods 282, 61–68.
Khoshnevis, M., Carozzo, C., Brown, R., Bardiès, M., Bonnefont-Rebeix, C., Belluco, S., Nennig, C., Marcon, L., Tillement, O., Gehan, H., Louis, C., Zahi, I., Buronfosse, T., Roger, T., Ponce, F., 2020. Feasibility of intratumoral 165Holmium siloxane delivery to induced U87 glioblastoma in a large animal model, the Yucatan minipig. PLoS One 15, e0234772.
Larsen, M., Bjarkam, C.R., Ostergaard, K., West, M.J., Sorensen, J.C., 2004. The anatomy of the porcine subthalamic nucleus evaluated with immunohistochemistry and design-based stereology. Anat. Embryol. 208, 239–247.
Lillethorup, T.P., Glud, A.N., Alstrup, A.K.O., Mikkelsen, T.W., Nielsen, E.H., Zaer, H., Doudet, D.J., Brooks, D.J., Sørensen, J.C.H., Orlowski, D., Landau, A.M., 2018a. Nigrostriatal proteasome inhibition impairs dopamine neurotransmission and motor function in minipigs. Exp. Neurol. 303, 142–152.
Lillethorup, T.P., Glud, A.N., Alstrup, A.K.O., Noer, O., Nielsen, E.H.T., Schacht, A.C., Landeck, N., Kirik, D., Orlowski, D., Sørensen, J.C.H., Doudet, D.J., Landau, A.M., 2018b. Longitudinal monoaminergic PET imaging of chronic proteasome inhibition in minipigs. Sci. Rep. 8, 15715.
Lind, N.M., Moustgaard, A., Jelsing, J., Vajta, G., Cumming, P., Hansen, A.K., 2007. The use of pigs in neuroscience: modeling brain disorders. Neurosci. Biobehav. Rev. 31, 728–751.
Meidahl, A.C., Orlowski, D., Sorensen, J.C., Bjarkam, C.R., 2016. The retrograde connections and anatomical segregation of the gottingen minipig nucleus accumbens. Front. Neuroanat. 10, 117.
Menz, H.B., Latt, M.D., Tiedemann, A., Mun San Kwan, M., Lord, S.R., 2004. Reliability of the GAITRite walkway system for the quantification of temporo-spatial parameters of gait in young and older people. Gait Posture 20, 20–25.
Mikkelsen, M., Møller, A., Jensen, L.H., Pedersen, A., Harajehi, J.B., Pakkenberg, H., 1999. MPTP-induced Parkinsonism in minipigs: a behavioral, biochemical, and histological study. Neurotoxicol. Teratol. 21, 169–175.
Molinet-Dronda, F., Gago, B., Quiroga-Varela, A., Juri, C., Collantes, M., Delgado, M., Prieto, E., Ecay, M., Iglesias, E., Marín, C., Peñuelas, I., Obeso, J.A., 2015. Monoaminergic PET imaging and histopathological correlation in unilateral and bilateral 6-hydroxydopamine lesioned rat models of Parkinson's disease: a longitudinal in-vivo study. Neurobiol. Dis. 77, 165–172.
Moon, J.H., Kim, J.H., Im, H.J., Lee, D.S., Park, E.J., Song, K., Oh, H.J., Hyun, S.B., Kang, S.C., Kim, H., Moon, H.E., Park, H.W., Lee, H.J., Kim, E.J., Kim, S., Lee, B.C., Paek, S.H., 2014. Proposed motor scoring system in a porcine model of Parkinson's Disease induced by chronic subcutaneous injection of MPTP. Exp. Neurobiol. 23, 258–265.
Netzley, A.H., Hunt, R.D., Franco-Arellano, J., Arnold, N., Vazquez, A.I., Munoz, K.A., Colbath, A.C., Bush, T.R., Pelled, G., 2021. Multimodal characterization of Yucatan minipig behavior and physiology through maturation. Sci. Rep. 11, 22688.
Nielsen, M.S., Glud, A.N., Møller, A., Mogensen, P., Bender, D., Sørensen, J.C., Doudet, D., Bjarkam, C.R., 2016. Continuous MPTP intoxication in the Göttingen minipig results in chronic parkinsonian deficits. Acta Neurobiol. Exp. (Wars.) 76, 199–211.
Nielsen, M.S., Sørensen, J.C., Bjarkam, C.R., 2009. The substantia nigra pars compacta of the Göttingen minipig: an anatomical and stereological study. Brain Struct. Funct. 213, 481–488.
Peter, B., De Rijk, E.P., Zeltner, A., Emmen, H.H., 2016. Sexual maturation in the female Göttingen Minipig. Toxicol. Pathol. 44, 482–485.
Schramke, S., Schuldenzucker, V., Schubert, R., Frank, F., Wirsig, M., Ott, S., Motlik, J., Fels, M., Kemper, N., Hölzner, E., Reilmann, R., 2016. Behavioral phenotyping of minipigs transgenic for the huntington gene. J. Neurosci. Methods 265, 34–45.
Seo, J., Yeo, H.G., Park, J., Won, J., Kim, K., Jin, Y.B., Koo, B.S., Lim, K.S., Jeong, K.J., Kang, P., Lee, H.Y., Son, H.C., Baek, S.H., Jeon, C.Y., Song, B.S., Huh, J.W., Lee, D.S., Lee, S.R., Kim, S.U., Lee, Y., 2020. A pilot study on assessment of locomotor behavior using a video tracking system in minipigs. Exp. Anim. 69, 62–69.
Sorensen, J.C., Nielsen, M.S., Rosendal, F., Deding, D., Ettrup, K.S., Jensen, K.N., Jorgensen, R.L., Glud, A.N., Meier, K., Fitting, L.M., Moller, A., Alstrup, A.K., Ostergaard, L., Bjarkam, C.R., 2011. Development of neuromodulation treatments in a large animal model–do neurosurgeons dream of electric pigs? Prog. Brain Res. 194, 97–103.
Steinmüller, J.B., Bjarkam, C.R., Orlowski, D., Sørensen, J.C.H., Glud, A.N., 2021. Anterograde tracing from the göttingen minipig motor and prefrontal cortex displays a topographic subthalamic and striatal axonal termination pattern comparable to previous findings in primates. Front. Neural Circuits 15.
Thorup, V.M., Laursen, B., Jensen, B.R., 2008. Net joint kinetics in the limbs of pigs walking on concrete floor in dry and contaminated conditions. J. Anim. Sci. 86, 992–998.
Thorup, V.M., Tøgersen, F.A., Jørgensen, B., Jensen, B.R., 2007. Biomechanical gait analysis of pigs walking on solid concrete floor. Animal 1, 708–715.
Tora, M.S., Texakalidis, P., Neill, S., Wetzel, J., Rindler, R.S., Hardcastle, N., Nagarajan, P.P., Krasnopeyev, A., Roach, C., James, R., Bruce, J.N., Canoll, P., Federici, T., Oshinski, J.N., Boulis, N.M., 2020. Lentiviral vector induced modeling of high-grade spinal cord glioma in minipigs. Sci. Rep. 10, 5291.
Tvilling, L., West, M., Glud, A.N., Zaer, H., Sørensen, J.C.H., Bjarkam, C.R., Orlowski, D., 2021. Anatomy and histology of the Göttingen minipig adenohypophysis with special emphasis on the polypeptide hormones: GH, PRL, and ACTH. Brain Struct. Funct. 226, 2375–2386.
Ungerstedt, U., 1968. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur. J. Pharm. 5, 107–110.
Yang, Y., Zhang, K., Yin, X., Lei, X., Chen, X., Wang, J., Quan, Y., Yang, L., Jia, Z., Chen, Q., Xian, J., Lu, Y., Huang, Q., Zhang, X., Feng, H., Chen, T., 2020. Quantitative iron neuroimaging can be used to assess the effects of minocycline in an intracerebral hemorrhage minipig model. Transl. Stroke Res. 11, 503–516.