Technical Note: Effects of age and confinement on pupillary light reflex in sows[1]

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ABSTRACT: Pupillary reflex has been used as a method to examine psychological problems in human clinics and mental disease research. Intensive animal farming has been shown to lead to chronic stress resulting in depression; however, comparing with humans we lack an effective clinical method to clinically inspect these psychological problems in animals. The goal of this study was to investigate the effect of age and housing conditions (confined crates vs. group pens) on pupillary light reflex (**PLR**) of sows to explore whether PLR can be used as an effective way to measure the psychological state of farm animals. In total, 270 pregnant sows were selected for PLR testing and divided into 6 treatments (45 sows per treatment) of 2 different environments (group-housed pen and confined gestation crates) and 3 parities (first, third, and fifth parities). Six selected pupil parameters: 1) latency of the pupil constriction onset (**LAT**); 2) the percent of the constriction (**CON**); 3) average constriction velocity (**ACV**); 4) maximum constriction velocity (**MCV**); 5) average dilation velocity (**ADV**); and 6) time of 75% recovery after constriction (**T75**) were examined. The results showed that there was no difference found in these PLR parameters between the breeds ($P > 0.05$) but the significant effects were found on LAT, CON, ACV, and MCV by age ($P < 0.01$). The group-housed sows had significantly higher CON, ACV, and MCV than those in the confined crates ($P < 0.05$). In conclusion, the pupillary light reflex of the sows was not affected by breed but by age. The results also indicate that some of PLR parameters were sensitive to housing conditions and suggest that ACV and MCV have potential to be sensitive indicators in relation to the psychological problem of sows.

Key words: animal welfare, mental depression, psychological index, pupil reflex, sow

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INTRODUCTION

Pupil constriction following light stimulation is referred to as the pupillary light reflex (**PLR**) controlled primarily by the sympathetic and parasympathetic branches of the autonomic nervous system (**ANS**) (Laeng et al., 2012). Pupillary light reflex is not affected by emotional state or consciousness; thus, it is regarded in humans as a convincing parameter indicating some psychological problems or diseases, including depression (Bär et al., 2004) and anxiety (Bitsios et al., 1996).

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For example, the latency of the pupil reflex (**LAT**) is longer in patients with depression, whereas the average constriction velocity (**ACV**) is lower (Fan et al., 2009). The pupil constriction velocity in patients with anxiety was lower than that in control subjects (Bakes et al., 1990). Thus, pupillary reflex, i.e., the delay of pupil reflex latency and lower pupil constriction velocity, is closely related to mental or psychological disorders in humans. In addition, the pupillary light reflex is also associated with age in humans; as age increases, the LAT of the pupillary reflex increases, whereas ACV decreases (Chen et al., 2003; Boev et al., 2005). Conventional pupillary reflex studies are mostly confined in human clinical studies, whereas there are a few reports on livestock pupillary reflex studies (e.g., Clinical consciousness of stunning, Sandercock, et al., 2014; Mental depression, Bao, et al., 2013; Li et al., 2017).

In intensive pig farming, gestation sows under confined conditions are subjected to physiological and psychological stress throughout the production process (Damgaard et al., 2009). The affected sows usually display abnormal behaviors, such as vacuum chewing, which is linked to psychological distress (van der Staay et al., 2010). Bao et al. (2013) and Li et al. (2017) found that the pupillary light reflex time (constriction velocity) of sows in a confined crate environment was longer than the group-housed sows, suggesting that confined rearing is likely to affect the sow's psychological state. However, pupillary light reflex time which reflects constriction velocity does not completely describe the whole pupillary light reflection process. So other factors associated with pupil light reflection should also be carefully considered. So as similar to human clinical diagnosis, the pupillary reflex of animals may also have the potential to be an indicator of psychological problems or depression. We have not yet conducted a systematic research for the effect of breed, age, and environmental conditions on specific PLR parameters. The goals of this study were to investigate the characteristics of pupillary light reflex in long-term confined sows and tried to identify the relationship of the PLR characteristics to breed, age, and housing conditions, and the results of the study may help us to find a possible clinical method to measure animals' psychological state, e. g., chronic stress or depression.

Table 1. The definitions of the tested parameters of pupillary light reflex

Parameters	Definitions
CON	The percent of the constriction (MAX-MIN)/MAX, where MAX and MIN represent the diameter of the pupil before the constriction (MAX) and at the peak of the constriction (MIN)
LAT, s	Latency represents the time to onset of the constriction
ACV, m/s	The average constriction velocity
MCV, m/s	The maximum constriction velocity
ADV, m/s	The average dilation velocity
T75, s	The total time taken by the pupil to recover 75% of the initial resting pupil size after reaching peak constriction

MATERIALS AND METHODS

Animal Management

Animal care and treatment were complied with the standards described in the guidelines for the care and use of laboratory animals of the Northeast Agricultural University (NEAU-[2011]-9).

The experiment was conducted at NEAU University's experimental pig farm at A-cheng, Harbin, China. The sows were housed in 2 housing conditions: 1) group-housed pens and 2) gestation crates. In the pen condition, there were 144 sows housed in 18 pens for the formal test and 8 sows were housed in each pen with the stocking density of 3.18 m^2 per sow, whereas in the confined condition, 504 sows (360 sows for preliminary test and 144 sows for the formal test) were individually housed in gestation crates with an area of 1.08 m^2 per sow. All animals were raised in the same houses during the experimental period, and the temperature of the houses remained at 16 to 20 °C (18.2 ± 2.8 °C), and humidity was 64.0% to 69.0% (66.5 ± 3.5%). Each sow had free access to water. The sows were fed twice a day (06:00 a.m. and 13:30 p.m.) with DM: 15.12 MJ/kg and 14% crude protein. The amount of feed supplied daily was about 2.5 kg/sow. Daily management, such as health inspection and manure removal, was conducted daily. All experimental sows maintained in the same houses throughout the experiment.

PLR Test

PLR test was conducted using a portable medical pupilometer (PLR-200TM, NeurOptics, NeurOptics Inc., Irvine, CA) which can measure 6 PLR parameters at a time. The definitions of these 6 parameters are given in Table 1. To ensure the accuracy of PLR measurements, the test was carried out during 17:30 to 18:30 p.m. to ensure the ideal room illumination (<250 lux). All group-housed sows and confined sows were unrestrained during testing when they were lying during this time. Before the test was conducted, we trained all the sows to adapt to the lighting of the pupilometer. The test light intensity was set to 180 μw (Bao et al., 2013). Each test was measured 3 times per individual at 5-min interval between each repetitive test to ensure complete recovery of pupil dilation and adequate time to rest.

Preliminary Test

The test was taken to examine the effect of breeds (genetic background) on pupillary reflex of sows, and 360 sows were used, of which 120 sows (60 sows of Landrace breed and 60 sows of Yorkshire breed) were in their first parity, 120 sows (60 sows of Landrace and 60 sows of Yorkshire) were the third parity, and other 120 sows (60 sows of Landrace and 60 sows of Yorkshire) were the fifth parity, respectively. The result showed that breed did not affect these 6 PLR parameters ($P > 0.05$).

The Formal Test

According to the results of the preliminary study, there were no significant effect on all PLR parameters PLR (LAT, ACV, maximum constriction velocity [**MCV**], average dilation velocity [**ADV**], and time of 75% recovery after constriction [**T75**]) by breeds; therefore, 288 pregnant sows were selected regardless of breed and were evenly divided into 2 groups: 1) gestation crates and 2) group-housed pens balanced for parity (first, third, and fifth parities) in each group. Therefore, there was a 2 × 3 design consisting of 6 treatments with 48 sows per treatment.

Statistical Analysis

All data were processed by Excel 2007 (Microsoft Corporation, Redmond, WA) and analyzed by Statistical Product and Service Solutions (SPSS, Version 22.0, IBM, Armonk, NY). All data were subjected to the Kolmogorov–Smirnov Test for examination of normality and homogeneity of variance test (chi-square analysis with frequent procedure). The data of ADV and T75 were conducted for cosine and logarithmic transformation, respectively, prior to ANOVA analysis. The effect of breeds on PLR parameters (the percent of the constriction [**CON**], LAT, ACV, MCV, ADV, and T75) was analyzed by one-way ANOVA. The effects of environment and age on PLR parameters were subjected to Factorial–ANOVA analysis. Considered the interaction of main effects, different environments and parities (ages) were set as the 2 main effects. The differences between the effects were analyzed with Duncan's multiple range test. $P < 0.05$ was considered as significant. All the results are provided as the means ± SD.

RESULTS

The Preliminary Test

As shown in Table 2, no breed effect was found in all PLR indicators, including LAT, ACV, MCV, ADV, and T75 (all P values > 0.05, except $P_{\text{CON of the fifth birth}} = 0.04$).

The Formal Test

The results of the effects of housing conditions on PLR parameters are given in Table 3. The results showed that the group-housed sows had significantly higher CON, ACV, and MCV or lower ADV than the confined sows ($P < 0.05$), but no effect was found for LAT and T75 ($P > 0.05$). The results also showed that age affected all these parameters, and as an increase in age, CON, ACV, MCV, ADV, and T75 significantly decreased ($P < 0.01$) while LAT increased ($P < 0.01$).

As shown in Table 3, the results suggested that as age increased, the confined condition affected CON more than the grouped housing condition, in which the confined sows showed significantly lower

Table 2. Effects of breed on PLR parameters (n = 60, mean ± SD)

		CON	LAT, s	ACV, m/s	MCV, m/s	ADV, m/s	T75, s
First birth	Landrace	0.165 ± 0.048	0.346 ± 0.111	2.046 ± 0.739	4.044 ± 1.015	0.833 ± 0.824	2.146 ± 0.825
	Yorkshire	0.159 ± 0.057	0.355 ± 0.127	2.012 ± 0.891	3.822 ± 0.956	0.869 ± 0.279	1.958 ± 0.908
Third birth	Landrace	0.124 ± 0.047	0.473 ± 0.170	1.756 ± 0.811	3.245 ± 1.027	0.641 ± 0.342	1.678 ± 0.818
	Yorkshire	0.128 ± 0.053	0.429 ± 0.141	1.913 ± 0.714	3.487 ± 0.924	0.757 ± 0.489	1.788 ± 0.972
Fifth birth	Landrace	0.084 ± 0.051^{y}	0.637 ± 0.243	1.277 ± 0.737	2.524 ± 1.059	0.554 ± 0.451	1.329 ± 0.949
	Yorkshire	0.102 ± 0.040^{x}	0.539 ± 0.176	1.495 ± 0.549	2.777 ± 0.763	0.653 ± 0.394	1.460 ± 0.827
Average	Landrace	0.118 ± 0.059	0.487 ± 0.220	1.698 ± 0.822	3.279 ± 1.200	0.708 ± 0.597	1.614 ± 0.941
	Yorkshire	0.120 ± 0.055	0.440 ± 0.167	1.809 ± 0.763	3.367 ± 0.983	0.692 ± 0.396	1.738 ± 0.923

Different superscripts, x and y, indicate a significant difference in column at $P < 0.05$.

Table 3. Effects of parity and housing condition on PLR parameters (n = 48, mean ± SD)

Parameters	Housing conditions	First parity	Third parity	Fifth parity	Mean
CON	Confined	0.16 ± 0.04^{a}	0.11 ± 0.05^{b}	0.08 ± 0.05^{cy}	0.12 ± 0.06^{y}
	Group housed	0.17 ± 0.04^{a}	0.13 ± 0.05^{b}	0.12 ± 0.05^{bx}	0.14 ± 0.05^{x}
	Average	0.16 ± 0.04^{a}	0.12 ± 0.05^{b}	0.10 ± 0.05^{c}	
LAT, s	Confined	0.36 ± 0.15^{c}	0.48 ± 0.18^{b}	0.65 ± 0.25^{ax}	0.50 ± 0.23
	Group housed	0.39 ± 0.11^{b}	0.44 ± 0.17^{b}	0.52 ± 0.23^{ay}	0.45 ± 0.18
	Average	0.38 ± 0.13^{c}	0.46 ± 0.18^{b}	0.59 ± 0.25^{a}	
ACV, m/s	Confined	1.96 ± 0.73^{ay}	1.68 ± 0.83^{a}	1.24 ± 0.73^{by}	1.62 ± 0.81^{y}
	Group housed	2.32 ± 0.77^{ax}	1.79 ± 0.64^{b}	1.61 ± 0.69^{bx}	1.91 ± 0.76^{x}
	Average	2.14 ± 0.77^{a}	1.73 ± 0.74^{b}	1.42 ± 0.73^{c}	
MCV, m/s	Confined	3.70 ± 0.99^{ay}	3.34 ± 0.99^{a}	2.50 ± 1.14^{by}	3.20 ± 1.16^{y}
	Group housed	4.14 ± 0.99^{ax}	3.47 ± 1.05^{b}	3.31 ± 1.03^{bx}	3.63 ± 1.09^{x}
	Average	3.92 ± 1.01^{a}	3.41 ± 1.02^{b}	2.90 ± 1.15^{c}	
ADV, m/s	Confined	0.93 ± 0.93^{ax}	0.71 ± 0.34^{ab}	0.60 ± 0.50^{by}	0.75 ± 0.65^{x}
	Group housed	0.74 ± 0.32^{y}	0.67 ± 0.34	0.64 ± 0.24^{x}	0.68 ± 0.30^{y}
	Average	0.83 ± 0.70	0.70 ± 0.33	0.62 ± 0.39	
T75, s	Confined	1.81 ± 0.90^{a}	1.50 ± 0.84^{ab}	1.29 ± 0.92^{b}	1.53 ± 0.90
	Group housed	1.50 ± 0.70	1.64 ± 0.81	1.47 ± 0.82	1.53 ± 0.78
	Average	1.65 ± 0.81^{a}	1.57 ± 0.83^{ab}	1.38 ± 0.87^{b}	

Different superscripts, a, b and c, indicate a significant difference in row at $P < 0.05$.
Different superscripts, x and y, indicate a significant difference in column at $P < 0.05$.

CON or higher LAT than the grouped sows at the fifth parity ($P < 0.05$) but no difference found in CON and LAT between the housing conditions at the first and third parities ($P > 0.05$). However, ACV, MCV, and ADV did not show regular patterns, where a significant difference was found between the housing conditions at the first and fifth parities ($P < 0.05$), but not at the third parity ($P > 0.05$).

There was no interaction effect between age and housing condition on CON, LAT, ACV, MCV, and T75 ($P > 0.05$), except for ADV ($P = 0.02$).

DISCUSSION

In the preliminary test, the result of no significant effect by breed on all PLR parameters may indicate that the pupillary light reflex is not affected by genetic variety. In human clinical research, it has been demonstrated that CON of patients with Alzheimer's disease and Parkinson's disease is lower than that of healthy individuals (Fotiou et al., 2009). According to the definition of CON, higher CON refers to smaller pupil constriction diameter or larger pupil rest diameter, indicating abnormal PLR, which may be caused by neuromuscular junction dysfunction or its normal PLR reaction induced by aging. Since the previous study has shown that the constriction and dilation ability of the pupil decreases with age (Li et al., 2013), Smith and Smith (1999) reported that LAT is found to be prolonged with age. Chen et al. (2003) demonstrated the same trend in ACV: the pupil constriction velocity of humans decreases with age. In this study, the CON, ACV, and MCV of the sows in both housing conditions were found to be decreased with parity, whereas LAT showed the opposite trend to CON, ACV, and MCV, which was similar to a report of humans (Radhakrishnan and Charman, 2007). These results suggest that sows or animals may have a similar physiological basis to humans in respect to pupillary light reflex controlled by the sympathetic and parasympathetic nerves of the autonomic nervous system (Koc et al., 2005). In humans, neuronal damage and synaptic decline will lead to gradual decline in neurological function with the increase in age (Houck and Person, 2015). Therefore, the results of the decline of PLR reflex function with increasing age in our study are consistent with the other research and support the above conclusion in human. In addition, McLaughlin et al. (2007) reported that chronic stress could lead to nerve tissue change in rats, and previous studies have shown that degenerative changes occur in optic nerve fibers and are manifested as thinning of retinal nerve fiber layer (**RNFL**) in depressed patients with Alzheimer's disease (AD) and other diseases (Moreno-Ramos et al., 2013). Chang et al. (2013) indicated that the PLR is strongly correlated with measurements of RNLF thickness, as thinner RNFL for each individual eye were associated with smaller response amplitude, slower velocity, and longer time to peak constriction and dilation after adjusting for age and sex. So, it can be inferred that the chronic stress which was induced by the restriction of environment may lead to decreased PLR.

Long-term confinement has proved to lead to increased vacuum chewing behavior in sows (Zhang et al., 2017), and it might indicate psychological frustration of the sows (Broom et al., 1995; Damgaard et al., 2009). The results of the different levels of ACV, MCV, and ADV between the confined crates and grouped pens at the same age (parity) might also indicate the influence of environmental conditions on PLR. As shown in the results of our study, as age increased, the velocity of constriction and dilation of the confined sows showed a sharper downward trend than the group-housed sows. This may suggest that the prolonged confinement even further reduced the velocity of constriction and dilation of pupil, indicating chronic stress response in the long-term confined sows (van der Beek et al., 2004). It further proves that the psychological problems may then affect PLR function (Mcdougal and Gamlin, 2010). Therefore, it can be argued that the decline in the velocity of pupil constriction can be indicative of psychological problems caused by chronic stress. Some evidences can be found in humans that PLR of the patients in depression was slower than that of healthy individuals with prolonged latency and lower velocity of constriction (Bär et al., 2004; Siegle et al., 2004). The studies on anxiety disorders have demonstrated that autonomic dysfunction affects the hypothalamus, vagal dorsal nucleus, and sympathetic ganglion (Kleiner and Marshall, 1987; Zesiewicz et al., 2003). Lv et al. (2016) reported a relationship between PLR and the changes in brain tissue lesions in pregnant sows, and specifically reported that PLR latency was significantly prolonged and accompanied by dissolution of hippocampus nerve cells as shown with hematoxylin-eosin staining. The decreased PLR with increased age under long-term confinement in these studies might be caused by degenerative changes to the nervous system in relation to emotional depression or psychological problems induced by chronic stress. Moreover, the results of our study in PLR responses, similar to that of human patients, may indicate that the long-term confinement of the sows may cause the sows to be as depressed or anxious as human patients and may be reflected with the prolonged PLR (Bär et al., 2004; Damgaard et al., 2009; Ray et al., 2009). Loewenfeld (1993) also reported a relationship between pupil light reflex and anxiety, which showed a decreased ACV associated with negative emotion. This is consistent with our results of ACV and MCV. Based on these results, we suggest that the contraction indicators of ACV and MCV were sensitive to environmental conditions and they may have the potential to be indicators of their psychological state, indicating depression or chronic stress. Of cause, this needs further research.

The results for ADV and T75 also showed changes with age and housing conditions, and the response of ADV was especially like that of ACV and MCV. However, we believed that ADV and T75 are not reliable indicators due to the limitation of the pupilometer, because it only allows 5 s recording during pupil dilation. Therefore, considering such a limitation of the pupilometer, we suggest that ACV and MCV are more suitable as indicative of psychological responses of sows than ADV and T75, and more investigation is needed.

CONCLUSION

The results of this study showed that pupillary light reflex (PLR) of sows were not affected by genetic breed but were likely related to age and environmental conditions, as the abnormal reactions in most of the PLR parameters were found to be associated with long-term confinement. ACV and MCV seem to show their potential as indicators of the psychological states of sows.

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