Behavioural Brain Research 401 (2021) 113080

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Regular touchscreen training affects faecal corticosterone metabolites and anxiety-like behaviour in mice

Viktoria Krakenberg [a,b,*], Maximilian Wewer [a], Rupert Palme [c], Sylvia Kaiser [a,b], Norbert Sachser [a,b], S. Helene Richter [a,b]

[a] Department of Behavioural Biology, University of Münster, Germany
[b] Otto Creutzfeldt Center for Cognitive and Behavioral Neuroscience, University of Münster, Germany
[c] Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

ARTICLE INFO

Keywords:
Touchscreen technology
Cognition research
Cognitive enrichment
Anxiety-like behaviour
Glucocorticoids
Mice

ABSTRACT

Automated touchscreen techniques find increasing application for the assessment of cognitive function in rodents. However, hardly anything is known about the potential impact of touchscreen-based training and testing procedures on the animals under investigation. Addressing this question appears particularly important in light of the long and intensive training phases required for most of the operant tasks. Against this background, we here investigated the influence of regular touchscreen training on hormones and behaviour of mice. Faecal corticosterone metabolites (FCMs), reflecting corticosterone levels around the time of treatment, were significantly increased in touchscreen-trained mice, even one week after the training phase was already terminated. Such an effect was not detected on baseline FCMs. Thus, regular touchscreen training can be assumed to cause long-term effects on hypothalamus-pituitary-adrenal axis activity. Furthermore, anxiety-like behaviour was increased in touchscreen-trained mice two weeks after the end of the training phase. Traditionally, this would be interpreted as a negative influence of the training procedure on the animals' affective state. Yet, we also provide two alternative explanations, taking the possibility into account that touchscreen training might have enriching properties.

Pre-clinical research in rodents has become essential for understanding the profound cognitive changes occurring across many human disorders, including for instance Alzheimer's disease or schizophrenia [1,2]. Inspired by touchscreen-based test procedures as they are routinely applied in human cognition research (e.g. CANTAB [3]), innovative touchscreen paradigms for the assessment of cognitive function have also been developed for rodents [2]. Such touchscreen tasks hold high translational potential, since several cognitive aspects of human disorders can be studied in rodents using analogues of the human paradigms [2,4,5]. Furthermore, they are featured by many automation-related advantages (e.g., reduction of experimenter influence, accurate data recording, high throughput of subjects [4]). However, despite their widespread usage, hardly anything is known about a potential impact of the touchscreen procedure itself on the physiology and behaviour of the animals under investigation. This is particularly surprising, as the respective operant tasks often require long and intensive training phases and hence are likely to have an influence on the experimental subjects. For example, training mice in a touchscreen task can last several weeks until months, a time during which the animals undergo daily training sessions of durations up to one hour [6].

A first study in mice examining the effects of regular touchscreen training on adrenocortical activity found significantly increased corticosterone levels in anticipation of training [7]. This provides initial evidence for a pronounced influence of the touchscreen training procedure, at least on an endocrinological level. Tying to this study, we here aimed to further unravel the impacts of daily touchscreen training on mice, extending our focus towards behavioural parameters. Thus, we analysed home cage activity of touchscreen trained and control mice, as well as their anxiety-like and exploratory behaviour in a battery of standardised tests. Additionally, we conducted an in-depth analysis of faecal corticosterone metabolites (FCMs), reflecting hypothalamus-pituitary-adrenal axis activity [8]. We hypothesised that touchscreen training would lead to alterations in both behavioural as well as endocrinological parameters assessed.

The study was conducted with 36 male C57BL/6J mice obtained from a professional breeder (Charles River Laboratories, Sulzfeld,

* Corresponding author at: Department of Behavioural Biology, University of Münster, Badestraße 13, 48149 Münster, Germany.
E-mail address: viktoria.krakenberg@uni-muenster.de (V. Krakenberg).

https://doi.org/10.1016/j.bbr.2020.113080
Received 20 August 2020; Received in revised form 30 November 2020; Accepted 14 December 2020
Available online 26 December 2020
0166-4328/© 2020 Elsevier B.V. All rights reserved.

Germany) at postnatal day (PND) 35. After arrival, mice were housed in groups of 3 animals per cage (Makrolon cages type III, 38 × 23 × 15 cm^3). The respective groups were treated as matched triplets during the following experimental phase. At PND 63, mice were transferred to single housing conditions to prevent any escalated aggression (for current discussions about male mouse housing see [9]). The experimental phase started at PND 69. Cages contained wood shavings as bedding material (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany), a wooden stick, a paper towel and a semi-transparent red plastic house (Tecniplast Deutschland GmbH, Hohenpeißenberg, Germany). Housing rooms were maintained at a reversed dark/light cycle with lights off at 08.00 a.m., a temperature of approximately 22 °C, and a relative humidity of about 50 %. Mice were provided with water and food (Altromin 1314; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) ad libitum, unless design-dependent restrictions of the daily food ration were applied during specific experimental stages (for details see below).

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Communities Council DIRECTIVE 2010/63/EU) and were approved by the local (Gesundheits- und Veterinäramt Münster, Nordrhein-Westfalen) and federal authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen "LANUV NRW").

The experimental design is depicted in Fig. 1. It comprised a *handling*, a *treatment* and a *behavioural test phase*. During the handling phase (experimental week 1), mice were accustomed to cup handling (c.f. [10]). During the subsequent treatment phase (experimental weeks 2–7), mice were subjected to one of the following three treatment groups: a touchscreen trained group (TS group, n = 12), a food restricted control group (FR, n = 12; food restriction is commonly required for touchscreen procedures, for details see below), or an ad libitum fed control group (AL, n = 12). The animals' home cage activity was recorded across experimental weeks 5–7. Starting with experimental week 8, all animals received ad libitum diet again. During the behavioural test phase comprising experimental weeks 10–12, mice were tested in a battery of tests on anxiety-like and exploratory behaviour. To study the effects of touchscreen training on adrenocortical activity, the animals' FCMs were monitored non-invasively across experimental weeks 1–9 [8]. Alternatingly, "baseline" and "reaction" values, the latter directly reflecting the time around treatment, were measured (for details see below).

The touchscreen group was set up to study the effects of touchscreen training. The term "touchscreen training" in this context does not only comprise potential effects of the cognitive training itself, but also potential effects of elements that can be considered as inherent to the touchscreen training procedure (e.g., regular food rewards, being removed from the home cage for a certain duration, etc.). TS mice experienced 5 touchscreen training sessions per week, each lasting 20 min. They were trained in an exemplary touchscreen paradigm, the *Visuomotor Conditional Learning* (VMCL) task, an established paradigm for the assessment of learning and memory in mice [11]. For a detailed description of the touchscreen task please see supplementary material. As touchscreen training is commonly combined with a restricted diet to increase the animals' motivation to work for food rewards (e.g [6,12].), TS mice were restricted to 90–95 % of their ad libitum feeding weights during the treatment phase (for details see supplementary material). To dissociate the effects of touchscreen training from those caused by food restriction, the two control groups (FR and AL) were deployed. FR mice were food restricted just as the TS mice, however, without experiencing touchscreen training. AL mice were fed ad libitum diet and did not receive touchscreen training either.

To monitor the animals' home cage activity during the treatment phase, behavioural observations took place at nine days across weeks 5, 6, and 7. Observations started approximately 3 h after treatment exposure. Each mouse was observed for 5 min per day via *focal animal sampling* and *continuous recording*, amounting to a total observation time of 45 min per animal. A mouse was considered as *active* when it showed any kind of motion, excluding tiny whisker, ear or tail movements [13].

During the behavioural test phase, anxiety-like and exploratory behaviour of the mice were assessed in the Elevated plus maze test (EPM; PND 132/133), Novel cage test (NC; PND 134/135), and Open field test (OF; PND 146/148). In addition, the degree of voluntary interaction with the experimenter was assessed in the Human-animal interaction test (HAI; PND 141; c.f. [13]). During testing, the experimenter was blind to the treatment of the mice. For detailed descriptions of the respective tests see supplementary material.

Across the experimental phase, "baseline" and "reaction" FCMs,

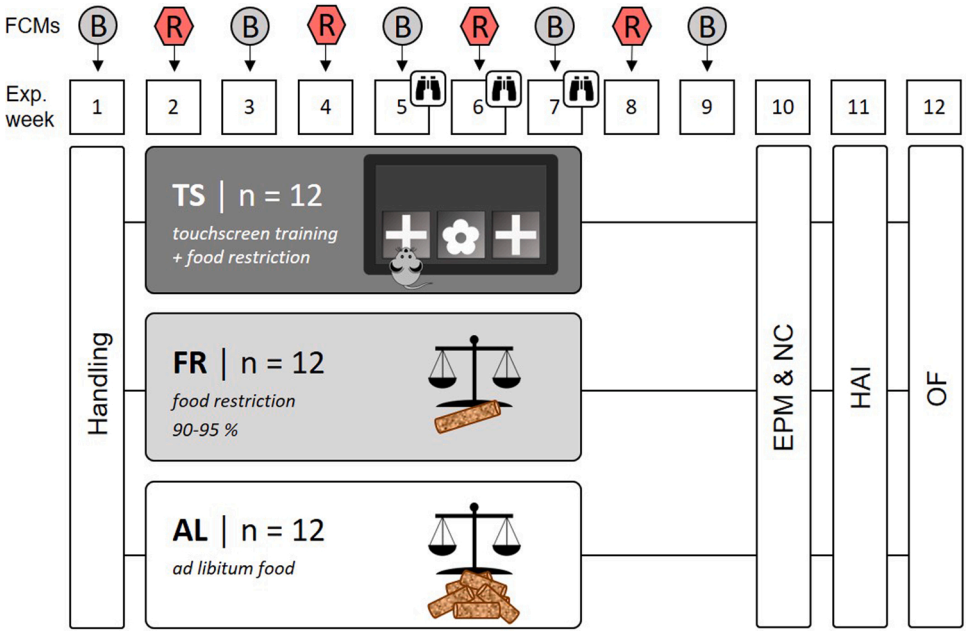

Fig. 1. Experimental Design. Handling phase (exp. week 1): mice were accustomed to cup handling (c.f. [10]). Treatment phase (exp. weeks 2-7): mice received one of three different treatments. The allocation to the treatment groups followed a randomised block design: triplets of mice that had been group-housed after arrival were randomly assigned to one of the treatment groups and henceforth constituted an experimental unit, matched with respect to rack position and the order of the experimental procedures (exception: the order of home cage observations was completely randomised). TS = touchscreen trained and food restricted (90-95 % of ad libitum feeding weights) group, FR = food restricted group (90-95 % of ad libitum feeding weights) without touchscreen training, AL = ad libitum fed group without touchscreen training. Behavioural test phase (exp. weeks 10-12): EPM = Elevated Plus Maze test, NC = Novel Cage test, HAI = Human-animal interaction test, OF = Open Field test. Home cage behaviour was observed during experimental weeks 5, 6, and 7. Faecal corticosterone metabolites (FCMs) were monitored weekly until experimental week 9. B (grey circles): "baseline" FCMs, R (red hexagons): "reaction" FCMs. Exp. = experimental.

respectively, were measured bi-weekly. “Baseline” FCMs reflect corticosterone levels during the time approximately 2 h after the daily treatment, i.e., when treatment effects can be assumed to have subsided again [7]. As during the dark phase, a peak of concentrations of FCMs in response to a treatment can be found 4–6 hours later [14], faeces for the assessment of “baseline” FCMs were collected approximately 5–8 h after the treatment. “Bbaseline” FCMs were obtained in experimental week 1 (= before the treatment phase), in weeks 3, 5, and 7 (= during the treatment phase), as well as in week 9 (= after the treatment phase). The values of weeks 1 and 9 allow for the detection of potential changes in “baseline” FCMs occurring after the start and the termination of the treatment phase, respectively.

Reaction” FCMs reflect corticosterone levels directly before, during, and after treatment. Taking into account the delay between treatment and the associated peak of concentrations of FCMs [14], samples were collected within 3–6 h after a mouse had received its respective treatment. "Reaction" FCMs were measured in experimental weeks 2, 4, 6 (= during the treatment phase), and 8 (= after the treatment phase). FCMs of week 8 were sampled in order to detect whether a potential anticipation of touchscreen training is maintained even after the treatment phase itself. For a detailed description of sample collections and processing please see supplementary material.

For the statistical analysis, heteroscedasticity and normal distribution of residuals were examined graphically and by using the Shapiro-Wilk normality test. Although residuals of FCM reaction data deviated from normal distribution, parametric statistics were applied, since simulation studies suggest mixed-effect models to be relatively robust against violations of distributional assumptions [15,16]. The analysis of behavioural data, including home cage activity and the parameters of the behavioural tests, was conducted using a linear mixed-effect model (LMM) with “treatment” as fixed factor and “group cage” as random factor, followed by sequentially Bonferroni-corrected *post hoc* comparisons:

$$\text{Behaviour} \sim \text{treatment} + (1|\text{group cage})$$

FCM data were analysed using an LMM with “treatment” and “experimental week” as fixed factors, and “individual” and “group cage” as random factors, again followed by sequentially Bonferroni-corrected *post hoc* comparisons:

$$\text{FCMs} \sim \text{treatment} * \text{experimental week} + (1|\text{ group cage/individual})$$

Analyses were carried out using the statistical software R (version 3.6.3 [17]) and R studio (version 1.2.5033 [18]). Degrees of freedom were rounded to the nearest integer. Differences were considered as significant for $p \leq 0.05$. Whenever the sequential Bonferroni correction was used for multiple, pairwise comparisons, the significance level was adjusted accordingly.

The results of the FCM analysis are depicted in Fig. 2. The statistical analysis of “baseline” FCMs did not reveal a significant effect of treatment ($F_{(2,33)} = 0.54$, $p = 0.59$). Yet, there was a significant effect of the experimental week ($F_{(4,128)} = 7.98$, $p < 0.01$). As indicated by sequentially Bonferroni-corrected post hoc comparisons, “baseline” FCMs of week 7 were higher compared to those of all other weeks. Additionally, “baseline” values measured in week 9 were significantly lower compared to those of week 3 ($p < 0.008$ for the respective comparisons; please note that using the sequential Bonferroni correction for a total number of 10 pairwise comparisons, the smallest p-value has to be $\leq$ 0.001 for an effect to be significant at the 0.05 level). No significant treatment-by-week interaction ($F_{(8,128)} = 0.63$, $p = 0.75$) could be detected.

In contrast to the “baseline” levels, “reaction” FCMs were significantly influenced by the treatment ($F_{(2,33)} = 4.17$, $p = 0.02$), with TS mice showing higher “reaction” values compared to AL mice ($p < 0.01$, significant after sequential Bonferroni correction for 3 pairwise comparisons). Furthermore, “reaction” values were also influenced by the experimental week ($F_{(2,99)} = 2.79$, $p = 0.04$). As revealed by post hoc testing, they increased from experimental week 6–8 ($p < 0.01$, significant after sequential Bonferroni correction for 6 pairwise comparisons). Adding to this, there was a significant treatment-by-week interaction ($F_{(2,99)} = 2.93$, $p = 0.01$), showing that the effect of treatment on “reaction” FCMs was most pronounced in experimental weeks 4 and 8 ($p <$ 0.01 for both comparisons, significant after sequential Bonferroni correction for 12 pairwise comparisons).

Regarding the analysis of the behavioural data, no significant effects of treatment could be detected on the animals’ home cage activity (LMM, $F_{(2,22)} = 0.24$, $p = 0.79$). In contrast to this, effects of treatment on anxiety-like and exploratory behaviour as assessed by the EPM and OF were apparent. Concerning the EPM, the time spent on the open arms of the apparatus ($F_{(2,22)} = 6.49$, $p < 0.01$), the number of entries made into these ($F_{(2,33)} = 5.19$, $p = 0.01$), as well as the distance travelled there ($F_{(2,22)} = 4.08$, $p = 0.03$) were influenced by the treatment (Table 1). Post hoc analyses indicated that TS mice were characterised by higher levels of anxiety-like and lower levels of exploratory behaviour, since they spent less time on the open arms and entered them less often compared to both FR and AL mice ($p \leq 0.017$ for the respective comparisons, significant after sequential Bonferroni correction for 3 pairwise comparisons; Table 1; Fig. 3A). Moreover, TS mice travelled significantly shorter distances on the open arms compared to AL mice ($p = 0.013$, significant after sequential Bonferroni correction for 3 pairwise comparisons; Table 1). In the OF, the number of entries made into the centre of the arena ($F_{(2,33)} = 5.88$, $p < 0.01$) and time the mice spent there ($F_{(2,22)} = 04.07$, $p = 0.03$) were influenced by treatment (Table 1).

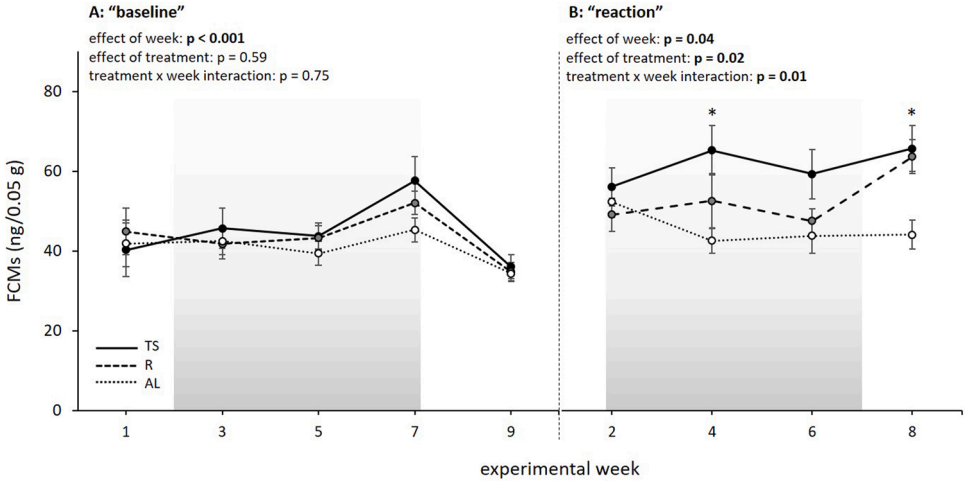

Fig. 2. Faecal corticosterone metabolites (FCMs). Grey area highlights the treatment phase. TS: touchscreen-trained mice, FR: food-restricted mice, AL: ad libitum fed mice. Data are presented as means ± SEM. Sample sizes: n = 12/group. Exception: experimental week 7, where $n_{TS} = 9$, $n_{FR} = 11$, $n_{AL} = 12$. (A) “Baseline” values, reflecting glucocorticoid levels approximately 2 h post treatment; Statistics: LMM. (B) “Reaction values”, reflecting glucocorticoid levels directly around the time of treatment. Statistics: LMM, sequentially Bonferroni-corrected post hoc tests for the significant interaction. *$p < 0.004$, significant at the 0.05 level after sequential Bonferroni correction for 12 pairwise comparisons.

Table 1
Statistical analysis of behavioural test parameters. Data are presented as untransformed means for the three groups (TS = touchscreen-trained mice, FR = food-restricted mice, AL = ad libitum fed mice) ± SEM. Statistical information given: main effects of treatment (LMM: F-ratio, p-value), unadjusted p-values of pairwise post hoc comparisons. Please note that using the sequential Bonferroni correction for three pairwise comparisons, the smallest p-value has to be ≤ 0.017 for an effect to be significant at the 0.05 level. For the statistical analysis, the latency to enter the half with hand (Human-animal interaction test) was square root transformed. Sample sizes: $n_{TS} = n_{R} = n_{AL} = 12$, bold: p-values indicating significant effects.

Parameter	TS	FR	AL	LMM		p-values of *pairwise comparisons*, unadjusted		
	Mean±SEM	Mean±SEM	Mean±SEM	F	p	AL vs. FR	AL vs. TS	FR vs. TS
Elevated plus maze test								
Time spent on open arms (rel.)	31.9 ± 2.6	43.0 ± 2.3	40.9 ± 2.2	6.485	**0.006**	0.516	**0.012**	**0.003**
Entries into open arms (rel.)	34.3 ± 2.3	42.7 ± 2.3	43.5 ± 1.8	5.193	**0.011**	0.816	**0.008**	**0.014**
Distance travelled on open arms (m)	2.8 ± 0.3	3.5 ± 0.2	2.9 ± 0.1	4.081	**0.031**	0.043	0.580	**0.013**
Sum of entries (#)	31.7 ± 1.2	30.0 ± 1.1	28.4 ± 1.2	1.921	0.163	0.350	0.063	0.326
Novel cage test								
Rearings (#)	68.8 ± 3.6	69.5 ± 4.2	69.9 ± 4.3	0.027	0.973	0.930	0.820	0.888
Open field test								
Time spent in centre (s)	17.7 ± 1.7	27.9 ± 3.5	24.6 ± 2.0	4.066	**0.031**	0.367	0.074	**0.011**
Entries into centre (#)	12.1 ± 0.8	17.9 ± 1.8	17.6 ± 1.0	5.880	**0.007**	0.863	**0.009**	**0.006**
Distance travelled (m)	42.2 ± 2.0	40.0 ± 1.7	39.0 ± 1.5	0.789	0.463	0.705	0.233	0.408
Human-animal interaction test								
Latency to enter half with hand (s)	3.0 ± 0.7	2.7 ± 0.6	3.9 ± 0.8	0.658	0.528	0.300	0.378	0.874
Time spent in half with hand (rel.)	36.5 ± 1.3	35.8 ± 2.0	36.3 ± 3.5	0.027	0.973	0.881	0.939	0.822
Entries into half with hand (#)	7.2 ± 0.5	6.6 ± 0.5	6.2 ± 0.3	1.277	0.292	0.514	0.126	0.364

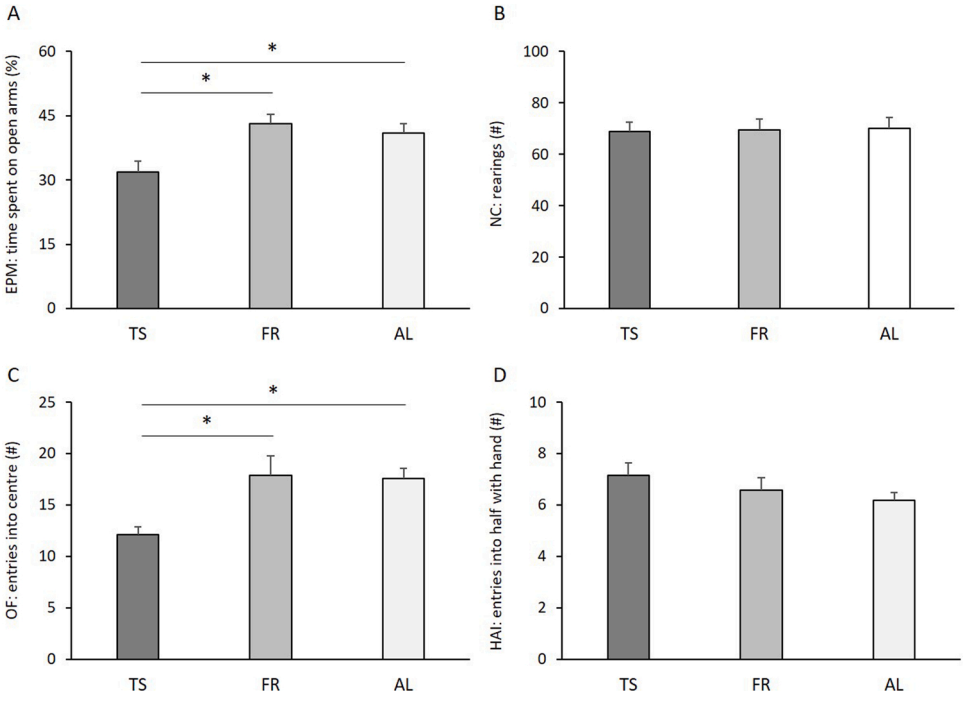

Fig. 3. Anxiety-like and exploratory behaviour. (A) Time spent on open arms of Elevated plus maze (EPM), (B) number of rearings in the Novel cage test (NC), (C) number of entries into the centre of the Open field test (OF), and (D) number of entries into the half with hand in the Human-animal interaction test (HAI). TS: touchscreen-trained mice, FR: food-restricted mice, AL: ad libitum fed mice. Sample sizes: n = 12/group. Data are presented as means + SEM. Statistics: LMM, sequentially Bonferroni-corrected post hoc tests. *p ≤ 0.017; please note that using the sequential Bonferroni correction for 3 pairwise comparisons, the smallest p-value has to be ≤ 0.017 for an effect to be significant at the 0.05 level.

Again, TS mice displayed higher levels of anxiety-like and lower levels of exploratory behaviour compared to FR and AL controls, as indicated by a fewer number of centre entries compared to both control groups ($p < 0.017$ for both comparisons, significant after sequential Bonferroni correction for 3 pairwise comparisons; Table 1; Fig. 3C), and a shorter time spent in the centre compared to FR animals ($p = 0.01$, significant after sequential Bonferroni correction for 3 pairwise comparisons; Table 1). However, no significant treatment effects could be found for the parameters obtained in the NC and HAI test (Fig. 3B, D; for statistical details see Table 1). A summary of the statistical details of all behavioural parameters analysed is given in Table 1.

In light of the growing popularity of touchscreen-based approaches for studying cognitive function in rodents, we here investigated the so far largely unexplored influence of this method on hormones and behaviour in mice.

Regarding the hormonal analysis, TS mice showed increased "reaction" FCMs. This is in line with the previously mentioned study by Mallien and colleagues, who report increased corticosterone concentrations in direct anticipation of a training session [7]. The time of direct training anticipation was also reflected in the "reaction" values obtained here. Interestingly, the training effect on "reaction" values was particularly pronounced in experimental week 8 (i.e., after the treatment phase), indicating that training anticipation might be maintained even beyond the actual training period. The lack of training effects on "baseline" FCMs also matches the results of Mallien and colleagues, who neither detected differences in corticosterone levels between touchscreen-trained and control mice 90 min after training [7]. Thus, taken together, regular touchscreen training can be assumed to cause fluctuations in HPA axis activity, with relatively higher HPA axis activity in TS compared to non-trained animals around the time of treatment.

Moreover, the lack of group differences in "baseline" FCMs is consistent with the animals' home cage activity recorded 2 h after the daily training sessions, for which also no significant group differences could be detected.

In the behavioural tests conducted, TS mice displayed increased levels of anxiety-like behaviour in the EPM and OF. In combination with the above discussed increase in HPA axis activity, these findings would traditionally be interpreted as indicating a negative influence of the touchscreen procedure on the animals' affective state [19]. This influence might for example have been conveyed via decreases in expected rewards, potentially leading to mild frustration during more difficult training steps [20].

However, this interpretation would be surprising in light of previous studies suggesting a beneficial impact of cognitive training by acting as enrichment [7,21,22]. In this regard, the increased anxiety-like behaviour of TS mice in the EPM and OF might also derive from a negative contrast effect. The idea of contrast effects influencing behavioural test outcomes has recently been discussed in a study investigating the effects of reward and punisher experiences on decision making [23]. It is based on the assumption that a discrepancy between an individual's anticipation of an event and the actually occurring event may cause a disappointment-like state, if the actual event is perceived as less rewarding than the expected one [23,24]. Thus, transporting the mice to another room to test them in the EPM or OF instead of placing them into the touchscreen chambers might have been contradictory to their expectations, and presumably less rewarding. In this regard, it also seems possible that effects of TS training on behaviour were only detected in the EPM and OF, conducted in a separate testing room, and not in the HAI or NC which were carried out in the animals' housing room.

A third possible explanation for our findings requires to consider one important aspect of the experimental design: behavioural testing took place two weeks after the touchscreen training phase. If touchscreen training would indeed constitute cognitive enrichment for the mice, this enrichment would have been withdrawn after the termination of the treatment phase. Such a loss of enrichment can induce negative affective states [25,26], and could thus also explain the higher levels of anxiety-like behaviour displayed by TS mice in the present study.

Taken together, the present results underline that the regular exposition to touchscreen training exerts pronounced effects on mice. Yet, it needs to be taken into account that these effects might not be attributable to the cognitive aspect of training alone. Potentially, also other factors that are inevitably linked to the touchscreen procedure might have played a role. For example, TS mice regularly received rewards during the training phase, but not afterwards. Therefore, a potential loss-of-training effect also goes along with a loss of rewards, which might have contributed to the observed effects. Moreover, TS mice were regularly confronted with light as a mild punishment during the training phase. Thus, they learned to associate light with a lack of reward delivery. This might have influenced their behaviour in the EPM and OF, since these tests rely on the animals' aversion of brightly lit areas [27]. Moreover, TS mice were removed from their home cages for a longer time span than AL and FR animals and additionally were carried to another room to be trained. This could have contributed to the observed effects, as well.

To conclude, our data clearly confirm a pronounced influence of daily touchscreen training. This influence was not limited to the training phase itself: even afterwards, effects on FCMs and anxiety-like behaviour were present. Yet, there are different possible explanations for our findings, requiring thorough, hypothesis-driven future investigations to clarify the influence of touchscreen training on the affective state of mice. Furthermore, future studies are needed to disentangle the effects of different aspects of touchscreen training procedures, such as the effects of reward or punishment type. Regardless of the affective valence of touchscreen training, however, our findings point out the necessity to consider the impact of touchscreen training on the subjects when applying this method.

Author statement

S.K., N.S., and S.H.R. conceived the study. S.K., V.K., N.S., and S.H.R. designed the experiments. V.K. and S.H.R. supervised the project. V.K. trained M.W. in conducting the experiments. M.W. carried out the experiments. R.P. determined and analysed the hormonal data. V.K. conducted the statistical analysis of the data and wrote the initial draft of the manuscript. All authors critically revised the manuscript and gave final approval for publication.

Funding

The authors would like to thank the Ministry of Innovation, Science and Research of the state of North Rhine-Westphalia (MIWF) for supporting the implementation of a professorship for behavioural biology and animal welfare to S.H.R. (Project: "Refinement of Animal Experiments"). This work was further supported by a grant from the German Research Foundation (DFG) to S.H.R and to N.S. (44541416/SFB-TRR58, Project A01).

Declaration of Competing Interest

The authors declare to have no competing interests.

Acknowledgements

The authors thank Edith Ossendorf and Edith Klobetz-Rassam for excellent technical assistance.

References

[1] A. Shepherd, S. Tyebji, A.J. Hannan, E.L. Burrows, Translational assays for assessment of cognition in rodent models of Alzheimer's disease and dementia, J. Mol. Neurosci. 60 (3) (2016) 371–382, https://doi.org/10.1007/s12031-016-0837-1.

[2] T.J. Bussey, A. Holmes, L. Lyon, A.C. Mar, K.A.L. McAllister, J. Nithianantharajah, et al., New translational assays for preclinical modelling of cognition in schizophrenia: the touchscreen testing method for mice and rats, Neuropharmacology 62 (3) (2012) 1191–1203, https://doi.org/10.1016/j.neuropharm.2011.04.011.

[3] P.J. Fray, T.W. Robbins, B.J. Sahakian, Neuorpsychiatyric applications of CANTAB, Int. J. Geriatr. Psychiatry 11 (4) (1996) 329–336, https://doi.org/10.1002/(SICI)1099-1166(199604)11:4<329::AID-GPS453>3.0.CO;2-6.

[4] T.J. Bussey, T.L. Padain, E.A. Skillings, B.D. Winters, A.J. Morton, L.M. Saksida, The touchscreen cognitive testing method for rodents: how to get the best out of your rat, Learn. Mem. 15 (7) (2008) 516–523, https://doi.org/10.1101/lm.987808.

[5] J. Talpos, T. Steckler, Touching on translation, Cell Tissue Res. 354 (1) (2013) 297–308, https://doi.org/10.1007/s00441-013-1694-7.

[6] S.H. Richter, A.S. Vogel, K. Ueltzhöffer, C. Muzzillo, M.A. Vogt, K. Lankisch, et al., Touchscreen-paradigm for mice reveals cross-species evidence for an antagonistic relationship of cognitive flexibility and stability, Front. Behav. Neurosci. 8 (2014) 154, https://doi.org/10.3389/fnbeh.2014.00154.

[7] A.S. Mallien, R. Palme, J. Richetto, C. Muzzillo, S.H. Richter, M.A. Vogt, et al., Daily exposure to a touchscreen-paradigm and associated food restriction evokes an increase in adrenocortical and neural activity in mice, Horm. Behav. 81 (2016) 97–105, https://doi.org/10.1016/j.yhbeh.2016.03.009.

[8] R. Palme, Non-invasive measurement of glucocorticoids: advances and problems, Physiol. Behav. 199 (2019) 229–243, https://doi.org/10.1016/j.physbeh.2018.11.021.

[9] S. Kappel, P. Hawkins, M.T. Mendl, To group or not to group? Good practice for housing male laboratory mice, Animals 7 (2017) 12, https://doi.org/10.3390/ani7120088.

[10] J.L. Hurst, R.S. West, Taming anxiety in laboratory mice, Nat. Methods 7 (10) (2010) 825–826, https://doi.org/10.1038/nmeth.1500.

[11] A.E. Horner, C.J. Heath, M. Hvoslef-Eide, B.A. Kent, C.H. Kim, S.R.O. Nilsson, et al., The touchscreen operant platform for testing learning and memory in rats and mice, Nat. Protoc. 8 (10) (2013) 1961, https://doi.org/10.1038/nprot.2013.122.

[12] S.J. Bartko, I. Vendrell, L.M. Saksida, T.J. Bussey, A computer-automated touchscreen paired-associates learning (PAL) task for mice: impairments following administration of scopolamine or dicyclomine and improvements following donepezil, Psychopharmacology 214 (2) (2011) 537–548, https://doi.org/10.1007/s00213-010-2050-1.

[13] J. Feige-Diller, V. Krakenberg, L. Bierbaum, L. Seifert, R. Palme, S. Kaiser, et al., The effects of different feeding routines on welfare in laboratory mice, Front. Vet. Sci. 6 (2020) 479, https://doi.org/10.3389/fvets.2019.00479.

[14] C. Touma, N. Sachser, E. Möstl, R. Palme, Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice, Gen. Comp. Endocrinol. 130 (3) (2003) 267–278, https://doi.org/10.1016/S0016-6480(02)00620-2.
[15] U. Knief, W. Forstmeier, Violating the normality assumption may be the lesser of two evils, bioRxiv (2018) 498931, https://doi.org/10.1101/498931.
[16] H. Schielzeth, N.J. Dingemanse, S. Nakagawa, D.F. Westneat, H. Allegue, C. Teplitsky, et al., Robustness of linear mixed-effects models to violations of distributional assumptions, Methods Ecol. Evol. 11 (9) (2020) 1141–1152, https://doi.org/10.1111/2041-210X.13434.
[17] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, 2015. Available online at: https://www.R-project.org/ (accessed March 27, 2020).
[18] R Studio Team, RStudio: Integrated Development for R. Boston, RStudio. Inc., MA, 2016. Available online at: http://www.rstudio.com/ (accessed March 27, 2020).
[19] E.S. Paul, E.J. Harding, M. Mendl, Measuring emotional processes in animals: the utility of a cognitive approach, Neurosci. Biobehav. Rev. 29 (3) (2005) 469–491, https://doi.org/10.1016/j.neubiorev.2005.01.002.
[20] J.E. Taylor, B. Ficzere, J.S. Louis, T.J. Schoenfeld, Examining the effects of exercise on frustration-induced anxiety-like behavior in rats, Psi Chi J. Psychol. Res. 24 (4) (2019).
[21] V. Krakenberg, V.T. von Kortzfleisch, S. Kaiser, N. Sachser, S.H. Richter, Differential effects of serotonin transporter genotype on anxiety-like behavior and cognitive judgment bias in mice, Front. Behav. Neurosci. 13 (2019) 263, https://doi.org/10.3389/fnbeh.2019.00263.
[22] G. Manteuffel, J. Langbein, B. Puppe, From operant learning to cognitive enrichment in farm animal housing: bases and applicability, Anim. Welf. 18 (1) (2009) 87–95.
[23] V. Neville, J. King, I.D. Gilchrist, P. Dayan, E.S. Paul, M. Mendl, Reward and punisher experience alter rodent decision-making in a judgement bias task, Sci. Rep. 10 (1) (2020) 1–14, https://doi.org/10.1038/s41598-020-68737-1.
[24] O.H. Burman, R.M. Parker, E.S. Paul, M. Mendl, Sensitivity to reward loss as an indicator of animal emotion and welfare, Biol. Lett. 4 (4) (2008) 330–333, https://doi.org/10.1098/rsbl.2008.0113.
[25] B.L. Smith, C.E. Lyons, F.G. Correa, S.C. Benoit, B. Myers, M.B. Solomon, J. P. Herman, Behavioral and physiological consequences of enrichment loss in rats, Psychoneuroendocrinology 77 (2017) 37–46, https://doi.org/10.1016/j.psyneuen.2016.11.040.
[26] N. Latham, G. Mason, Frustration and perseveration in stereotypic captive animals: is a taste of enrichment worse than none at all? Behav. Brain Res. 211 (1) (2010) 96–104, https://doi.org/10.1016/j.bbr.2010.03.018.
[27] J.N. Crawley, What's Wrong with My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice, John Wiley & Sons, 2007.