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Short communication

Mu opioid receptor knockout mice in the Morris Water Maze: A learning or motivation deficit?

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Abstract

An earlier study done by Kas et al. [Kas MJ, van den Bos R, Baars AM, Lubbers M, Lesscher HM, Hillebrand JJ, et al. Mu-opioid receptor knockout mice show diminished food-anticipatory activity. Eur J Neurosci 2004;20(6):1624–32] suggested that mu opioid receptor (MOR) knockout mice have deficits in the motivational component rather than in the information processing component of learning. To substantiate this difference further, MOR knockout mice and wildtype littermates were tested in the Morris Water Maze (MWM), which allows for testing both components of learning. On traditional parameters for performance, no significant differences between genotypes were found. However, swimming velocity, indicative of motivation, decreased for MOR knockout mice during the course of training but not for wildtype mice. In contrast, probe trial performance was comparable between genotypes. Again, these results suggest normal information processing abilities but a decreased motivation in this MOR knockout mouse. Our conclusion is discussed in the light of other studies using MOR knockout mice that do find differences on information processing between genotypes in MWM performance.

Keywords: Mice; Morris Water Maze; Learning; Motivation; MOR; Exon-1; Mu

Learning relies on two major functional systems, motivation (reward related) and information processing (acquisition, consolidation and retrieval of a behavioural response). Deficits in learning, for instance in mutant mice, may be ascribed to either of these systems. To assess gene–behaviour relationships, genetically modified animals are tested in various set-ups. Mu opioid receptor (MOR) knockout mice have been tested in pain, addiction, and conditioning tests [e.g. 26,13,23]. MOR knockout mice were also studied in set-ups in which more than one system is tested, such as motivation and learning. Previous research using a Food Anticipatory Activity protocol [11] showed that MORs are involved in anticipatory behaviour using exon-1 MOR knockout mice and littermate wildtypes. Two findings emerged. First, exon-1 MOR knockout mice did develop anticipatory behaviour per se prior to a fixed time window of food availability. Second, compared to wildtype mice, MOR knockout mice displayed less anticipatory activity prior to a fixed time window of food availability. In other words, acquisition and consolidation of information is shown to be intact in these mice, while the motivational component of learning seems disturbed. The former is known to be hippocampus dependent [e.g. 27]. However, motivation is thought to be dependent on the meso-accumbens dopamine system, in particular the activational aspects of motivation, such as measured by anticipatory behaviour or instrumental behaviour [e.g. 7, 8, 21,25]. To delineate this finding in MOR knockout mice further, we used the Morris Water Maze (MWM) in which both the hippocampus [17] and the meso-accumbens dopamine system [19] are involved.

Traditionally used parameters for performance in the MWM are "latency to reach the platform" and "distance moved". In the present experiment we attempt to dissociate two aspects of learning by using distinct parameters for each component: (1) information processing and (2) motivation. The result of information processing is best measured during a probe trial in which the hidden platform is removed from the swimming pool [5]. Animals that have learned the location of the hidden platform in relation to spatial cues spent more time in the quadrant where the platform

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was previously located than in any of the other quadrants, i.e. they show spatial bias or spatial memory [16]. On the other hand, motivational aspects of learning are best measured by parameters that are not affected by spatial components. Other behavioural tests often measure motivation by reward approach velocity [e.g. 1,4]. In the MWM, which is a negatively reinforced task, reaching the platform is considered rewarding. Therefore, motivation to reach this reward is best measured by "mean swimming velocity" during trials. "Latency to reach the platform" cannot be used as specific measure for either component since deficits in either spatial learning or motivation will lead to changes in this parameter. Given the earlier study of Kas et al. [11], we hypothesised that exon-1 MOR knockout mice show intact information processing but a reduced motivation for a reward; thus, knockouts will have equal performance in the probe trial, but will have a lower "mean swimming velocity" to the platform than wildtype mice.

To test our hypothesis, we used 15 naive homozygote female littermate mice ($n = 8$ exon-1 MOR knockout mice; $n = 7$ wildtypes, this line was created by Schuller et al. [24]) aged 3–4 months. A stable background was ensured by regularly backcrossing to the background C57BL/6JOlaHsd strain (Harlan, the Netherlands). Mice were housed in same sex littermate-groups (2–3 animals per cage, Makrolon® type II, Techniplast, Italy). Tap water and laboratory chow were available *ad libitum*. Sawdust bedding was used. In addition a tissue, shelter and paper shreds were provided. After weaning at day 22–25, the animals were housed under the same housing conditions in another mixed sex room, again under a reversed day–night regimen (07.00 h–19.00 h red light, 19.00 h–07.00 h white light) and constant temperature (21 ± 1 °C). All handling was done under red light conditions. Testing occurred between 10.00 h and 16.00 h. The Ethical Committee of Utrecht University approved of the experiment.

The protocol used in the present study was based on the original protocol for rats [17] with some modifications for mice [6]. We used a circular white pool (40 cm high) filled to a height of 24.5 cm (diameter at water level 135 cm) with tap water that was kept at 25 °C (±1 °C). Water was refreshed on a daily basis. The maze was located in a room equipped with large black and white cues on three of its walls and another cue hanging from the ceiling. A transparent Perspex cylinder (diameter 10 cm) was placed 0.5 cm below the water surface in the centre of one of the quadrants. A camera was attached above the pool and connected to a computer. On training days (1–4), two sessions each consisting of two trials, were conducted. The morning session was conducted between 10.00 h and 12.30 h, the afternoon session between 13.30 h and 16.00 h. For practical reasons the afternoon session of day 1 consisted of only one trial. The starting point shifted one quadrant every following trial. A trial ended when the mouse climbed onto the platform or when the maximum time of 120 s had passed. On the fifth day, the platform was removed from the pool and a probe trial of 90 s was conducted. Probe trials all started at the same quadrant, opposite from the platform quadrant. Video tracks were analysed using Ethovision® (Noldus Information Technology, The Netherlands), sampling at a rate of 12.5 samples per second. For training trials (day 1–4), "latency to reach the platform" as well as "distance moved" and "mean swimming velocity" were recorded. The latter parameter was measured only when mice were swimming (velocity > 7.7 cm/s). Movements at lower velocity were considered to be floating, which may be a different strategy based on a different brain system [3]. This data analysis therefore allows us to correctly analyse possible motivational differences between genotypes. Daily scores were averaged (3–4 trials). During the probe trial it was determined how much time mice spent in the quadrant where the platform was previously positioned ("time spent in target quadrant"). SPSS 12.0 software was used to perform statistics. Data over days were analysed using a General Linear Model (GLM) test for repeated measures with the between subject factor "genotype" and within factor "days". When appropriate, post hoc comparisons were done between genotypes using one-way ANOVA. The probe trial was analysed using a one-sample *t*-test. Unless otherwise indicated all statistics are two-tailed. *p*-Values smaller than or equal to 0.05 are considered significant; $0.05 < p \leq 0.10$ is considered a trend and $p > 0.10$ is considered not significant.

Independent of genotype, "latency to reach the platform" decreased over days: all mice needed progressively less time to move from the starting point to the hidden platform over days (Fig. 1, factor days: $F_{(3,39)} = 7.6$, $p \leq 0.001$). No significant genotype effect was found. Independent of genotype, "distance moved" decreased over days: all mice showed increasingly shorter pathways from the starting point to the hidden platform over days (Fig. 2A, factor days: $F_{(3,39)} = 14.76$, $p \leq 0.001$). A tendency to significance was found between genotypes (factor genotype: $F_{(1,13)} = 3.438$, $p \leq 0.087$). Independent of genotype, the "proportion of time spent swimming" decreased over days (data not shown, factor days: $F_{(3,39)} = 2.935$, $^{*}p < 0.045$). No significant genotype effect was found. In order to achieve normal distribution a square root transformation was performed for "mean swimming velocity". The data in Fig. 2B show that wildtype mice swam with a constant velocity across days while knockout mice decreased their velocity across days. This was confirmed by statistical analysis as we found a significant interaction term (days × genotype: $F_{(3,39)} = 4.541$, $p \leq 0.008$). In addition repeated measurements per genotype showed that wildtypes did not change mean swimming velocity over days (factor days: $F_{(3,18)} = 0.858$, $p \leq 0.480$), while knockout mice significantly decreased their mean swimming velocity (factor days: $F_{(3.21)} = 4.793$, $p \leq 0.011$). Post hoc analysis per day revealed

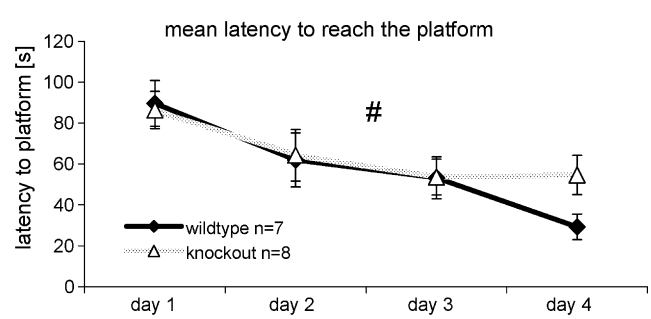

Fig. 1. Effects of exon-1 MOR deletion during MWM training; "Latency to reach the platform". Shown are means (±S.E.M.) of daily averages of 3–4 trials $^{\#}p \leq 0.05$ significant differences over days (further statistics see text).

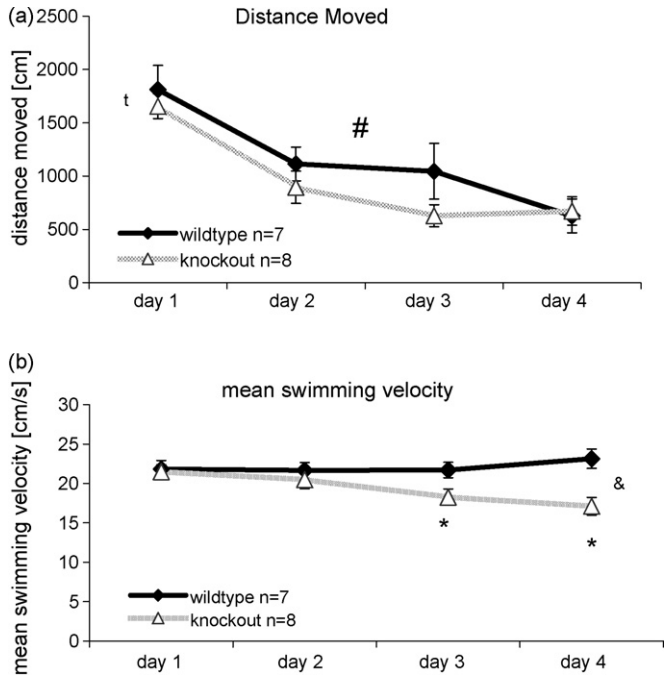

Fig. 2. Effects of exon-1 MOR deletion during MWM training; "distance moved" (A) and "mean swimming velocity" (B). Shown are means (±S.E.M.) of daily averages of 3–4 trials. $^{\#}p \leq 0.05$ significant differences over days, $^{\dagger}p \leq 0.1$ for a trend between genotypes, $^{\&}p \leq 0.05$ for day × genotype effect and $^{*}p \leq 0.05$ for significant differences between genotypes per day (further statistics see text).

that significant differences between genotypes were present on day 3 (one-way ANOVA $F_{(1,14)} = 5.534$, $p \leq 0.035$) and day 4 (one-way ANOVA $F_{(1,14)} = 12.402$, $p \leq 0.004$). Finally, during the probe trial mice of both genotypes had proportionally higher "time spent in target quadrant" scores than can be expected based on chance (Fig. 3, one tailed one sample *t*-tests versus 25%; for wildtype mice $t = 3.277$, d.f. = 6, $p < 0.009$; for MOR knockout mice $t = 2.053$, d.f. = 7, $p \leq 0.04$). Between genotypes no differences existed for these proportions (Fig. 3, one-way ANOVA $F(1,14) = 0.071$, $p \leq 0.794$).

On classical Morris Water Maze parameters ("latency to reach the platform" and "distance moved") we did not find significant differences between MOR knockout mice and wildtype mice. However, on "mean swimming velocity" we did observe differences between MOR knockout mice and wildtype

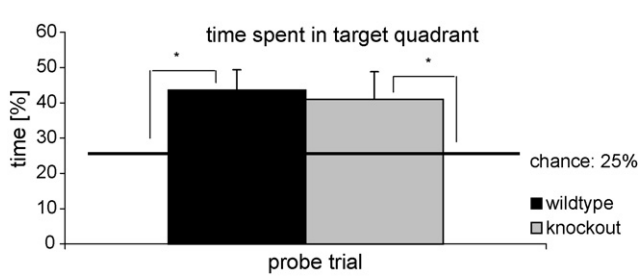

Fig. 3. Effects of exon-1 MOR deletion during MWM probe-trial; "time spent in target quadrant". Shown are percentages of the maximum trial duration of 90 s. $^{*}p \leq 0.05$, tested against chance level (25%), one-tailed (further statistics see text).

mice. In particular "mean swimming velocity" was significantly decreased on day 3 and 4 in MOR knockout mice. This effect is thought to be a result of decreased motivation in these mice.

Normal probe trial behaviour was shown in this knockout mouse, i.e. both genotypes significantly preferred the target quadrant. The parameters together suggest that exon-1 MOR knockout mice have a deficit in the motivational component of learning rather than a learning deficit per se. It is therefore concluded that MOR knockout mice are less motivated but as accurate as wildtype mice to reach a reinforcing source.

We ascribe our results to a deficit in motivation, rather than to a motor problem since mice of different genotypes had equal performance for all parameters on the first day of training. This implicates that all mice were able to swim in a coordinated way.

During the first training days no significant differences between genotypes were found on "mean swimming velocity". Therefore, we suggest that, independent of genotype, maximum motivation to reach the platform is present during these early trials. Although not investigated, fear (for drowning) could underlie this maximal motivation in both genotypes.

The decrease of "mean swimming velocity" for MOR knockout but not wildtype mice during the course of training indicates a declining motivation to reach the platform in MOR knockout mice and not in wildtype mice. As the mice become acquainted with the possibility to escape, fear is likely to diminish, thereby leaving room for other systems to underlie behaviour.

Overall the data are in line with Kas et al. [11] who showed that MOR knockout mice actually develop anticipatory behaviour but less so than wildtype mice. The observed behavioural differences between genotypes could be due to: differences in the perception of reward value or differences in activational aspects of motivation. Thus far we have not observed differences in wildtypes and knockout mice with respect to reward value (sucrose consumption and taste reactivity patterns, unpublished data). Therefore, we suggest that the organization of the appropriate response is affected in these mice. It has been shown that the role of the meso-accumbens dopamine system, which is involved in effort to collect rewards [22,25], becomes stronger over days in the MWM [19]. Mathon et al. [13] showed that MOR knockout mice show deficits in this meso-accumbens dopamine system. Accordingly deficits in the meso-accumbens dopamine system may thus explain our findings in the MOR knockout mice (cf. Ref. [11] anticipatory behaviour).

The present results are only partially in line with other studies in which MOR knockouts were tested in the MWM [9,10]. MOR knockouts in these studies displayed a longer "latency to reach the platform" than wildtype mice after at least 1 day of training. In our study, MOR knockout mice also tend to have a longer "latency to reach the platform" than wildtype mice. However, in contrast to our results, MOR knockout mice in these studies showed less "time spent in target quadrant" during the probe trial than wildtype mice. The main differences between their results on MOR knockout mice and ours are not likely to be explained by methodological differences, such as the use of pre-training trials, home cage housing conditions, lighting, test apparatus, gender, and background strain (see also Refs. [26,2,28]). If any, effects of these environmental aspects on Morris Water Maze behaviour

are likely to also affect the performance of wildtypes. Wildtypes in these and our MWM experiment have a comparable learning curve, i.e. "latency to reach the platform" decreased at least 50% over 15–16 trials. Therefore, we think that a comparison between results of MOR knockout mice in different experiments can be made.

To explain the differences between our experiments and those reported in the literature, we hypothesize, although speculative, that these differences are dependent on the *line* of MOR knockout that is used and thus dependent on the exon(s) that is/are knocked out. In the present study, exon-1 MOR knockout mice (described in Ref. [24]) were used, whereas Jang et al. [10] used exon-2 and -3 MOR knockout mice (described in Ref [12]) and Jamot et al. [9] used exon-2 MOR knockout mice (described in Ref. [14]). This hypothesis is supported by data from Silva et al. [25] who used antisense mapping. They found that mu agonists have opposite effects on behaviour dependent on which exon (exon 1 or exon 2 and 3) of the MOR gene is shut down. This segregation, exon-1 versus exon-2 (and exon-3), is in line with the different MOR knockout lines. In addition, it has been suggested that exon-1 is associated with mu-1 opioid receptors [20] but not with other mu opioid receptors. Accordingly one may suggest that exon-1 MOR knockout mice lack predominantly the mu-1 opioid receptors, whereas other lines may lack mu-2 or all mu opioid receptors. The hippocampus, on which spatial learning is largely dependent [17], has a higher density of mu-2 than of mu-1 receptors [18]. This may explain the intact spatial performance in our exon-1 knockout mouse.

It is clear that this hypothesis needs to be substantiated by further experiments, such as measurement of LTP in the hippocampus of exon-1 knockout mice. Matthies et al. [15] found deficits in LTP in hippocampus of exon-2 and-3 knockout mice.

In conclusion, by using a separate parameter indicative of motivation, a novel hypothesis on distinct effects of deletion of exon-1 MOR on the motivational component rather than information-processing component of learning was formulated.

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