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Cell type-specific genetic reconstitution of CB1 receptor subsets to assess their role in exploratory behaviour, sociability, and memory

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Abstract

Several studies support the notion that exploratory behaviour depends on the functionality of the cannabinoid type 1 (CB1) receptor in a cell type-specific manner. Mice lacking the CB1 receptor in forebrain GABAergic or dorsal telencephalic glutamatergic neurons have served as essential tools revealing the necessary CB1 receptor functions in these two neuronal populations. However, whether these specific CB1 receptor populations are also sufficient within the endocannabinoid system for wild-type-like exploratory behaviour has remained unknown. To evaluate cell-type-specific sufficiency of CB1 receptor signalling exclusively in dorsal telencephalic glutamatergic neurons (Glu-CB1-RS) or in forebrain GABAergic neurons (GABA-CB1-RS), we utilised a mouse model in which CB1 receptor expression can be reactivated conditionally at endogenous levels from a complete CB1-KO background. The two types of conditional CB1-rescue mice were compared with CB1 receptor-deficient [no reactivation (Stop-CB1)] and wild-type [ubiquitous reactivation of endogenous CB1 receptor (CB1-RS)] controls to investigate the behavioural consequences. We evaluated social and object exploratory behaviour in four different paradigms. Remarkably, the reduced exploration observed in Stop-CB1 animals was rescued in Glu-CB1-RS mice and sometimes even surpassed CB1-RS (wild-type) exploration. In contrast, GABA-CB1-RS animals showed the lowest exploratory drive in all paradigms, with an even stronger phenotype than Stop-CB1 mice. Interestingly, these effects weakened with increasing familiarity with the environment, suggesting a causal role for altered

Abbreviations: CB1, cannabinoid type 1 receptor; CB1-RS, complete CB1 rescue, equivalent to wild-type; DI, discrimination index; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; GABA-CB1-RS, CB1 rescue in GABAergic forebrain neurons; Glu-CB1-RS, CB1 rescue in dorsal telencephalic glutamatergic neurons; NORT, novel object recognition task; PFC, prefrontal cortex; RI, resident-intruder test; Stop-CB1, CB1 expression blocked by Stop-cassette.

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neophobia in the observed phenotypes. Taken together, using our genetic approach, we were able to substantiate the opposing role of the CB1 receptor in dorsal telencephalic glutamatergic versus forebrain GABAergic neurons regarding exploratory behaviour.

KEYWORDS

CB1 receptors, endocannabinoids, exploratory behaviour, GABA, glutamate

1 | INTRODUCTION

The endocannabinoid (eCB) system represents an essential regulatory mechanism modulating a multitude of physiological processes in the brain (Busquets-Garcia et al., 2018; Katona & Freund, 2012; Lutz et al., 2015). The central component of the eCB system is the cannabinoid type 1 (CB1) receptor, which is prominently located at synaptic terminals within the central nervous system. Its activation by endogenous ligands, referred to as eCBs, leads to a suppression of neurotransmitter release. Excitatory cortical glutamatergic and inhibitory GABAergic neurons express the CB1 receptor at significant levels (Busquets-Garcia et al., 2018; Katona & Freund, 2012; Lutz et al., 2015). Thus, by controlling the release of these neurotransmitters, a functional eCB system can maintain an optimal excitation state of the brain.

In several pathological states, e.g., in autism, anxiety disorders, and schizophrenic-like disorders, the balance between excitatory and inhibitory transmission has been suggested to be dysregulated (Amitai et al., 2012; Cristino et al., 2020; Navarrete et al., 2020; Sala et al., 2011). Furthermore, a common finding in these pathologies is an alteration of exploratory behaviour, a phenotype that can also be observed in conditional mutants lacking the CB1 receptor in dorsal telencephalic glutamatergic or forebrain GABAergic neurons (Häring et al., 2011; Helfand et al., 2017; Jacob et al., 2009; Lafenêtre et al., 2009; Terzian et al., 2014). Moreover, the two CB1 receptor-expressing cell populations were found to be responsible for the opposing biphasic behavioural effects induced by cannabinoid treatment regarding for example anxiety-like, fear-coping, and novelty-seeking behaviour (Lafenêtre et al., 2009; Metna-Laurent et al., 2012; Rey et al., 2012). The fact that the anxiety and emotional response of an individual is essential for its exploratory behaviour underlines the importance of a balanced signalling between GABAergic and glutamatergic neurotransmission.

The conditional knock-out mice described above have served as valuable tools to understand the necessity of the eCB system in dorsal telencephalic glutamatergic or forebrain GABAergic neurons (Busquets-Garcia et al., 2018; Lutz et al., 2015; Marsicano et al., 2003; Monory et al., 2006). Nevertheless, whether these sites of expression of the CB1 receptor are also sufficient within the endocannabinoid system for physiological brain functions is still unclear. A modified approach allows the analysis of this relevant question (Ruehle et al., 2013). In this genetic model, "rescue" mice were generated that express the CB1 receptor exclusively in dorsal telencephalic glutamatergic neurons (Glu-CB1-RS; Ruehle et al., 2013) or in forebrain GABAergic neurons (GABA-CB1-RS; Remmers et al., 2017), enabling us to evaluate the sufficiency of glutamatergic and GABAergic CB1 receptor signalling to allow behavioural performance comparable with that of wild-type animals (CB1-RS).

In the present study, we applied four different paradigms (open field, sociability, novel object recognition and resident-intruder test) to analyse different dimensions of exploratory behaviour. We used the aforementioned conditional rescue mouse lines and their CB1 deficient littermates (Stop-CB1; Ruehle et al., 2013), and included a wild-type control group with a ubiquitous CB1 receptor rescue (CB1-RS; Ruehle et al., 2013) to evaluate the behavioural potential of cell type-specific rescue of CB1 receptor expression. Interestingly, GABA-CB1-RS animals showed strongly reduced exploratory drive in all paradigms used, as compared with Stop-CB1 mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Experimental protocols were carried out in accordance with the Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes and approved by the Ethical Committee on animal care and use of Rhineland-Palatinate, Germany. Animals were housed in a temperature- and humidity-controlled room (22°C ± 1; 50% ±1) with a 12 hr light-dark cycle (lights on at 5 a.m.) and had access to food and water ad libitum. The study was performed on adult (3–4 months old) male mice of three different mouse lines: Glu-CB1-RS mice ($n = 21$) and their Stop-CB1 receptor-deficient littermates ($n = 19$), GABA-CB1-RS mice ($n = 17$) and their Stop-CB1 littermates ($n = 13$), and wild-type-like CB1-RS mice ($n = 36$). Due to the incidental death of some mice between test days or video tracking problems, the *n* numbers differ between the individual tests. Animals were single-housed one week prior to behavioural testing. Generation of

the Glu-CB1-RS, GABA-CB1-RS, and CB1-RS lines was performed by crossing Stop-CB1 mice (Ruehle et al., 2013) which carry a loxP-flanked stop cassette upstream of the endogenous CB1 receptor coding sequence with either NEX-Cre (Goebbels et al., 2006; Schwab et al., 2000), Dlx-Cre (Zerucha et al., 2000) or EIIa-Cre (Lakso et al., 1996) mice. All mutant lines were backcrossed for at least 10 generations into C57BL/6J background (Charles River, Germany). Genotyping was performed according to previous publications (Bellocchio et al., 2010; Remmers et al., 2017; Ruehle et al., 2013). For an overview of CB1 receptor expression patterns in the different mutant lines, see Table 1 and for their histological verification (see Gutiérrez-Rodríguez et al., 2017; Remmers et al., 2017; Ruehle et al., 2013; de Salas-Quiroga et al., 2015).

2.2 | Experimental design

The experimental design and execution were as described in Häring et al. (2011). Briefly, animals were group-housed (3–5 animals per cage type 2 (H14.0 × W20.5 × L26.5 cm), EBECO Germany) until one week before behavioural testing. Mice were then single housed to avoid behavioural differences between dominant and subordinate animals. The same test animals were used in each paradigm. Between the sociability and open field test as well as between the novel object recognition task (NORT) and resident-intruder test (RI), mice were allowed to rest for one week. Open field and NORT were performed on two consecutive days. All experiments were started one hour after turning off the lights (i.e., 6 p.m.), in the active phase of the animals, with only a minimal red light-source in the room. Under these conditions, our lux-metre measured 0 lx. Behaviour was recorded using an infrared detecting camera. In total, four different tests were performed in the following order:

2.2.1 | Sociability test

As described previously (Häring et al., 2011), the sociability test was performed following a modified protocol published by Moy et al. (2004). The test chamber (H41 cm × W42 cm × L70 cm) was divided into three compartments (H40 cm × W40 cm × L22 cm), linked by openings (H7.5 cm × W10 cm) in the dividing walls. Test animals were placed in the middle compartment. Male group-housed C57BL/6N animals (10–12 weeks old) served as interaction partners.

Habituation Phase

The test animal was placed into the middle compartment for 5 min with entries to the side compartments blocked.

Social exploration phase

The animal tested was exposed to two cylindrical cages (10 cm in diameter; 30 cm high [upper 20 cm Plexiglas, lower 10 cm covered by metal bars 1 cm apart to allow interaction but prevent fighting]) for 10 min. Each cylinder was positioned in one of the two side compartments. One cage contained an unknown C57BL/6N interaction partner (mouse), whereas the other one was empty (object). After each test mouse, the position of the interaction partner (left versus. right compartment) was alternated to avoid any bias. Time exploring the mouse and the object were scored manually (see 2.3 behavioural scoring). The discrimination index (DI) was then calculated as the difference between the time spent exploring the mouse (A) and the object (B) divided by the total time exploring both [(A - B)/(A + B)]. A positive DI which is significantly different from zero indicates a preference for the mouse, whereas a negative DI indicates a preference for the object. To minimise the stress levels of the animals used as interaction partners, they were habituated to the cages four times for 10 min distributed over 2 days prior to the actual test days.

Social Memory Phase: Two hours after the social exploration phase, an additional unknown interaction partner (novel) was introduced for 10 min. The interaction partner from the social exploration phase (familiar) was again placed into the same cage and the same compartment as before. The novel animal was placed into the former empty cage and positioned in the compartment of the former empty cage. The DI was calculated as the difference between the time spent exploring the novel mouse (A) and the familiar mouse (B) divided by the total time exploring both [(A - B)/(A + B)]. Here, a positive or negative DI which is significantly different from zero is considered to reflect the retention of memory.

2.2.2 | Open field

The test was performed in a white plastic open field chamber (H40 cm x W40 cm x L40 cm). Animals were placed into the arena and allowed to explore it freely for 10 min. The horizontal

TABLE 1 Summary of the different CB1 receptor mutant mice used

	Stop-CB1	CB1-RS	Glu-CB1-RS	GABA-CB1-RS
CB1 receptor rescue	None	Ubiquitous	Dorsal telencephalic glutamatergic neurons	Forebrain GABAergic neurons
CB1 receptor depletion	Ubiquitous	None	All other cell types	All other cell types

distance covered by the animals was recorded and divided into exploration in the centre (13 x 13 cm) and the periphery. Vertical exploration (rearing and jumping) was scored manually by an observer blind to the genotype of the animals.

2.2.3 | Novel object recognition task

The protocol used was modified from Ennaceur and Broadbent et al. (2010), Delacour (1988), Tang et al. (1999), and Tordera et al. (2007) and performed in the same box that was used for the open field test. The task itself was divided into three different phases.

Habituation phase

Animals were placed into the empty open field and allowed to explore the box for 10 min once a day for 2 days. Locomotion behaviour was recorded during the first habituation session and reported as the open field test described above.

Object exploration phase

On day 3, two identical objects (O1 left and O1 right; two metal cubes with H4 cm × W3 cm × L5 cm) were placed symmetrically 6–7 cm from the walls and separated 16–18 cm from each other (in two adjacent corners). The mouse was placed into the box at an equal distance from both objects and allowed to explore freely for 10 min. Time exploring the objects was scored manually (see 2.3 behavioural scoring). The discrimination index (DI) was then calculated as the difference between the time spent exploring O1 right (A) and O1 left (B) divided by the total time exploring both [(A - B)/(A + B)]. A positive DI which is significantly different from zero indicates a preference for O1 right, whereas a negative DI describes a preference for O1 left.

Object memory phase

Two hours after the first exposure to the object, the mouse was again placed into the open field and exposed to the familiar object (O1 left) and to a novel object (O2 right). O2 right was a plastic billiard ball (5.72 cm in diameter) fixed on a metal plate (0.2 cm). O1 left was always positioned on the left side, while O2 right was on the right side. The mouse was allowed to explore freely for 10 min. The DI was calculated as the difference between the time spent exploring O2 right (A) and O1 left (B) divided by the total time exploring both [(A - B)/(A + B)]. A positive or negative DI which is significantly different from zero is considered to reflect the retention of memory.

2.2.4 | Resident-intruder test

The RI was performed by placing an unknown group-housed C57BL/6N intruder into the home cage (H14.0 cm × W20.5 cm × L26.5 cm) of the test animal for 5 min. To decrease interaction induced by the intruder, younger animals (males, 12–14 weeks) were used. Interaction was defined as any type of physical contact (except fighting) and clearly directed towards the partner. Also fighting onset was evaluated.

2.3 | Behavioural scoring

For all experiments (except the RI, which was performed in the home cage), chambers and cages were cleaned with 70% ethanol after each trial to avoid olfactory cues. Experiments were video-recorded using an infrared detecting camera, and the total time that the test animals spent directly interacting with object or interaction partner was manually evaluated by trained observers blind to the genotype of the mice. Locomotion during the social exploration and social memory phase of the sociability test as well as during the open field test was measured by Panlab's SMART 2.5.5 video-tracking software (Barcelona, Spain) and EthoVision 8.5 (Noldus; Wageningen, Netherlands). To counterbalance the individual differences of the interaction partners, they were equally used for test mice of different genotypes. Exploration was defined as the orientation of the nose directly to the object or interaction partner at a distance <1 cm and/or touching the object/interaction partner with the nose and whiskers. Time spent climbing and sitting on the object was not regarded as exploration, and was, therefore, excluded from measurement (Tang et al., 1999).

2.4 | Statistical analysis

Data are presented as mean ± standard error of the mean (*SEM*). Statistics were generated by IBM SPSS Statistics Software for Windows (version 22; Chicago, IL, USA). Differences were considered significant at $p < 0.05$. Repeated-measures ANOVAs with genotype, test phase, interval, and object as independent variables were initially used to analyse the sociability test, open field, and NORT. Similarly, repeated-measures ANOVAs with genotype, test phase and interval as independent variables were applied to evaluate the DIs. Since, indeed, several interactions were detected (data not shown), all following analyses were performed on split data (for test phase and interval). Univariate ANOVA with genotype as the independent variable was used to evaluate interaction, DIs and locomotion. Possible confounding factors, such as the number of occurrences that a test mouse climbed on an object during the NORT were initially included as covariates for the measurement of object interaction in the object memory phase of the NORT. Significant genotype

effects (towards CB1-RS and Stop-CB1) were further analysed using Tukey's-HSD post hoc test for multiple comparisons, or simple effects with Sidak correction in case of significant time effects. When the assumption of homogeneity of variances was not met, the Welch correction and Games-Howell multiple comparison procedure were applied instead. Additionally, in order to evaluate whether DIs deviated significantly from zero, we used One-sample *t*-test comparing to reference value zero. Rescue was considered "complete" if the genotype of interest (Glu-CB1-RS or GABA-CB1-RS) showed a significant difference from Stop-CB1 and no significance compared with CB1-RS. Values intermediate between Stop-CB1 and CB1-RS (with significance from either both or none), were considered as "partly sufficient." No difference from (or more extreme values than) Stop-CB1 together with a significant difference from the CB1-RS group was interpreted as "no sufficiency" of the rescue.

3 | RESULTS

In order to improve the readability of the text, statistical details are mainly implemented in table form in this manuscript (Table 2–5, S1–S6). Since initial repeated-measures ANOVAs of the sociability test and the NORT (interaction time and DI) revealed several interactions between genotype, test phase, interval, and object, data were analysed separately for the separate test phases and intervals. Furthermore, since the strongest preference scores tend to occur early in the test phase (Broadbent et al., 2010), we present only the first 5-min intervals in the main Figures and the second 5-min intervals in the Supporting Information.

3.1 | Exploratory behaviour

3.1.1 | Locomotion

The evaluation of locomotor activity in the open field revealed that Glu-CB1-RS mice covered a larger distance over the 10-min trial than CB1-RS controls (Table 2; Figure 1a). Analysis per minute revealed that both Glu-CB1-RS and Stop-CB1 mice had higher initial locomotion than CB1-RS, significant only during the first 3 min (Figure 1b). Time spent in the centre was similar between the CB1-RS, Stop-CB1, and Glu-CB1-RS groups, but was lower in GABA-CB1-RS than in Stop-CB1 mice, and did not change over the duration of the test (Figure 1c). Vertical exploration did not differ between the groups (Figure 1d).

Within the two 5-min intervals of the social exploration and social memory phase, no significant locomotion differences were detected between all genotypes (Table S1).

3.1.2 | Social exploration

When compared with the CB1-RS control group, Stop-CB1 animals displayed a significant decrease in the interaction time with their respective partners during the sociability test (Table 3; Figure 2a and S1a) and resident-intruder test. (Table 4; Figure 2b). The reactivation of CB1 receptor expression in glutamatergic neurons rescued this phenotype, as no difference in animate exploration could be observed between Glu-CB1-RS and CB1-RS mice in both paradigms (Tables 3 and 4; Figure 2a and b, and S1a). However, in the sociability test, the rescue was complete, since the performance of Glu-CB1-RS mice was significantly different from Stop-CB1

TABLE 2 Statistical analysis of locomotor performance in the open field test; Univariate ANOVA

Variable	Factor	*df*1	*df*2	*F*	*p*	post hoc G	*p*
Total distance	G	3	84	3.198	0.027	CB1-RS versus Glu-CB1-RS	0.019
Distance per minute	sphericity	44		153.677	<0.001		
	M	5.651	474.708	27.252	<0.001		
	M x G	16.954	474.708	2.503	<0.001	M1: CB1-RS versus Glu-CB1-RS	0.029
						M1: CB1-RS versus Stop-CB1	0.002
						M2: CB1-RS versus Stop-CB1	0.036
						M3: CB1-RS versus Stop-CB1	0.029
	G	3	84	3.198	0.027		
Centre time	G	3	41.159	5.945	0.002	Stop-CB1 versus GABA-CB1-RS	0.005
Vertical exploration	G	3	84	0.477	0.699	nd	

Note: G, genotype; M, time in minutes; nd, not determined.

TABLE 3 Statistical analysis of exploration and memory in the sociability test; Univariate ANOVA

Phase	Interval	Stimulus	Factor	*df*1	*df*2	*F*	*p*	post hoc G	*p*
Exploration	1st 5 min	Object	G	3	97	2.190	0.094	nd	
		Mouse	G	3	97	6.485	<0.001	Stop-CB1 versus Glu-CB1-RS	0.044
								CB1-RS versus GABA-CB1-RS	0.004
	2nd 5 min	Object	G	3	97	2.648	0.053	nd	
		Mouse	G	3	45	17.378	<0.001	Stop-CB1 versus CB1-RS	<0.001
								Stop-CB1 versus Glu-CB1-RS	0.001
								Stop-CB1 versus GABA-CB1-RS	0.029
								CB1-RS versus GABA-CB1-RS	0.012
Memory	1st 5 min	Familiar	G	3	97	11.089	<0.001	Stop-CB1 versus CB1-RS	<0.001
								Stop-CB1 versus Glu-CB1-RS	0.018
								CB1-RS versus GABA-CB1-RS	<0.001
		Novel	G	3	97	15.244	<0.001	Stop-CB1 versus CB1-RS	<0.001
								Stop-CB1 versus Glu-CB1-RS	<0.001
								CB1-RS versus GABA-CB1-RS	<0.001
	2nd 5 min	Familiar	G	3	97	9.506	<0.001	Stop-CB1 versus CB1-RS	<0.001
								Stop-CB1 versus Glu-CB1-RS	<0.001
								Stop-CB1 versus GABA-CB1-RS	0.030
		Novel	G	3	97	4.953	0.003	Stop-CB1 versus CB1-RS	0.004

Note: G, genotype; nd, not determined.

TABLE 4 Statistical analysis of interaction behaviour in the resident-intruder test; Univariate ANOVA

Variable	Factor	*df*1	*df*2	*F*	*p*	post hoc G	*p*
Resident-induced interaction	G	3	46	34.455	<0.001	Stop-CB1 versus CB1-RS	0.002
						Stop-CB1 versus GABA-CB1-RS	<0.001
						CB1-RS versus GABA-CB1-RS	<0.001
Intruder-induced interaction	G	3	94	1.360	0.260	nd	
Latency to first fight	G	3	97	0.801	0.496	nd	

Note: G, genotype; nd, not determined

mice in both intervals of the social exploration phase (Table 3; Figure 2a and S1a), whereas in the resident-intruder test the rescue was partial as Glu-CB1-RS performance was neither significantly different from that of the CB1-RS mice nor from that of Stop-CB1 animals (Table 4, Figure 2b). After showing a stronger reduction in expression than the Stop-CB1, rescuing the CB1 receptor expression in GABAergic neurons only partially rescued the performance towards that of wild-type CB1-RS mice with the progression of time during the sociability test (Table 3; Figure 2a and S1a).

In the resident-intruder test, GABA-CB1-RS mice again induced even less interactions with the intruder than Stop-CB1 animals (Table 4; Figure 2b). No differences were observed in the behaviour of the intruders towards the test animals of the different genotypes (Table 4; Figure S2a). In the majority of the trials, no fighting occurred and the latency to the first fight was not different between the groups (Table 4; Figure S2b).

3.1.3 | Object exploration

The analysis of both phases of the novel object recognition task revealed no behavioural differences in general object

TABLE 5 Statistical analysis of exploration and memory in the novel object recognition test; Univariate ANOVA

Phase	Interval	Stimulus	Factor	*df*1	*df*2	*F*	*p*	post hoc G	*p*
Exploration	1st 5 min	O1 left	G	3	42	10.374	<0.001	CB1-RS versus GABA-CB1-RS	0.007
								Stop-CB1 versus GABA-CB1-RS	0.018
		O1 right	G	3	40	9.545	<0.001	CB1-RS versus Glu-CB1-RS	0.044
								CB1-RS versus GABA-CB1-RS	0.014
								Stop-CB1 versus GABA-CB1-RS	0.018
	2nd 5 min	O1 left	G	3	81	7.921	<0.001	CB1-RS versus Glu-CB1-RS	0.030
			B	3	81	10.047	<0.001		
		O1 right	G	3	84	0.725	0.540	nd	
Memory	1st 5 min	O1 left	G	3	36	5.076	0.005	Stop-CB1 versus Glu-CB1-RS	<0.001
			B	3	81	4.891	0.004		
		O2 right	G	3	84	4.066	0.009	no sign. towards CB1-RS or Stop-CB1 mice	
	2nd 5 min	O1 left	G	3	36	3.417	0.027	no sign. towards CB1-RS or Stop-CB1 mice	
		O2 right	G	3	84	0.480	0.697	nd	

Note: B, batch; G, genotype; nd, not determined; O1, object 1; O2, object 2.

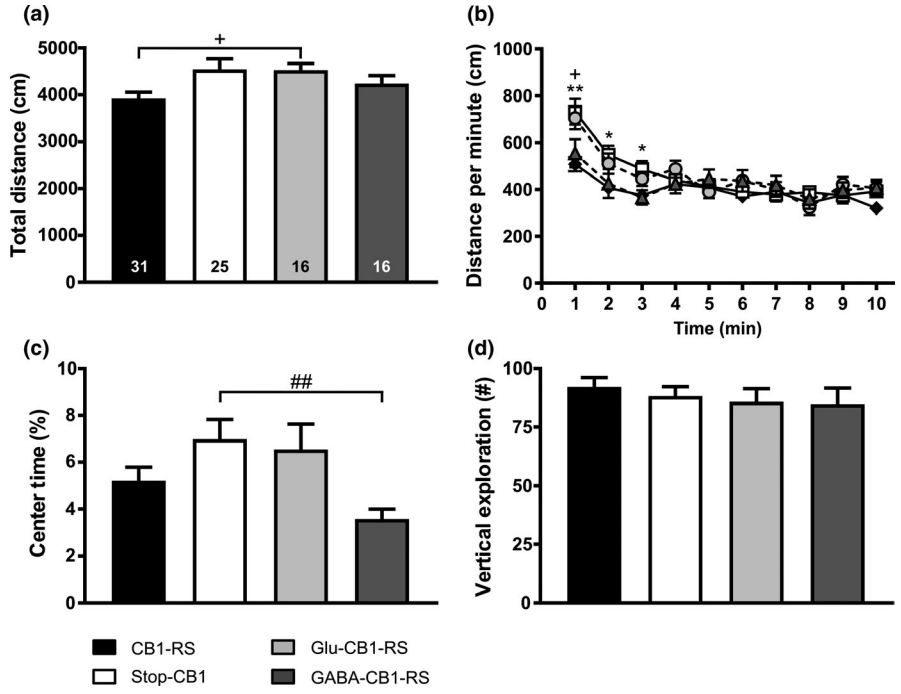

FIGURE 1 Effects of cell type-specific CB1 receptor rescue on locomotion and exploration in the open field test. Total horizontal exploration of an open field by mice of different genotypes expressing the CB1 receptor in specific neuronal subpopulations (a). Distance travelled per minute (b). Time spent in the centre (c) and vertical exploration (d) throughout the test. Bars represent the different genotypes; CB1-RS black (diamonds with solid line), Stop-CB1 white (squares with solid line), Glu-CB1-RS light grey (circles with dashed line), GABA-CB1-RS dark grey (triangles with dashed line). Graphs show mean values ± *SEM*. Numbers displayed in the bars indicate the number of animals per genotype (*n*) used in the open field test. Univariate or repeated-measures ANOVA followed by Tukey (a, c) or simple effects with Sidak correction (b). **p* < 0.05 Stop-CB1 versus CB1-RS, +*p* < 0.05 CB1-RS versus Glu-CB1-RS, ##*p* < 0.01 Stop-CB1 versus GABA-CB1-RS

exploration between CB1-RS and Stop-CB1 animals (Table 5; Figures 2c, 3b, S1, and S3b). Also when presented simultaneously with a social stimulus during the social exploration test, no significant differences were detected between the genotypes in exploring the empty cage (Table 3; Figure 2a and S1a). However, we detected significant changes in animals with a

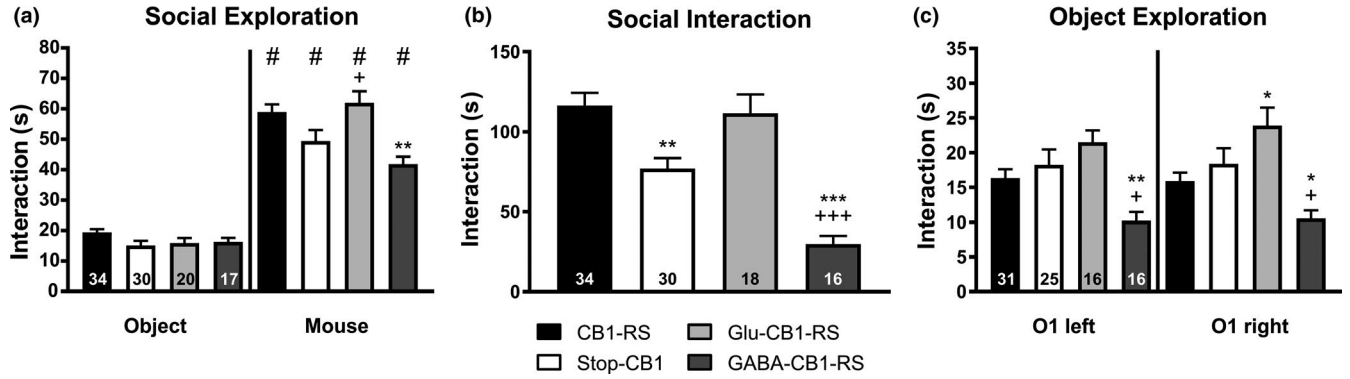

FIGURE 2 Effects of cell type-specific CB1 receptor rescue on exploration for the first 5-min interval. Comparison of animate (mouse) and inanimate (object) exploration during the social exploration phase (a). Social interaction with an unknown, younger intruder induced by the resident (b). Exploration of two identical objects (O1 left and O1 right) during the object exploration phase (c). Bars represent different genotypes. Graphs show mean values + *SEM*. Numbers displayed in the bars indicate the number of animals per genotype (*n*) used in each test. Univariate ANOVA: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (significantly different from CB1-RS); $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ (significantly different from Stop-CB1). $^{\#}$Animals display a significant preference for this interaction partner or object. O1, object 1; O2, object 2

specific rescue of CB1 receptor expression in glutamatergic neurons during the NORT. When compared with CB1-RS mice, Glu-CB1-RS mice showed an increase in the exploration of the objects (Table 5; Figure 2c and S1b). Similar to the social exploration, in the NORT GABA-CB1-RS animals also displayed a significant decrease in exploration which disappeared over time (Table 5; Figure 2c and S1b). The different genotypes did not show any preference for one of the objects (DI significantly different from 0) over the complete 10-min period. The only exception was found in CB1-RS mice, which showed a small but a significant preference for O1 right in the second 5-min interval (Table S2). When the complete 10-min period of the object exploration phase was analysed, this effect was not observed anymore. Thus, no substantial side preference was observed.

3.2 | Memory

3.2.1 | Social memory

In the social memory phase of the sociability test, Stop-CB1 mice in comparison with CB1-RS mice showed a significant decrease in the interaction time with partner animals during both intervals (Table 3; Figure 3a and S3a). In Glu-CB1-RS mice, this phenotype was completely rescued, as their performance in animate exploration was not significantly different from that of CB1-RS mice and differed from that of Stop-CB1 mice (Table 3; Figure 3a and S3a). Reactivation of the CB1 receptor expression in GABAergic neurons again displayed a time-dependent rescue of the wild-type phenotype (Table 3; Figure 3a and S3a).

In both intervals of the social exploration phase, all genotypes showed a clear preference (DI significantly different from

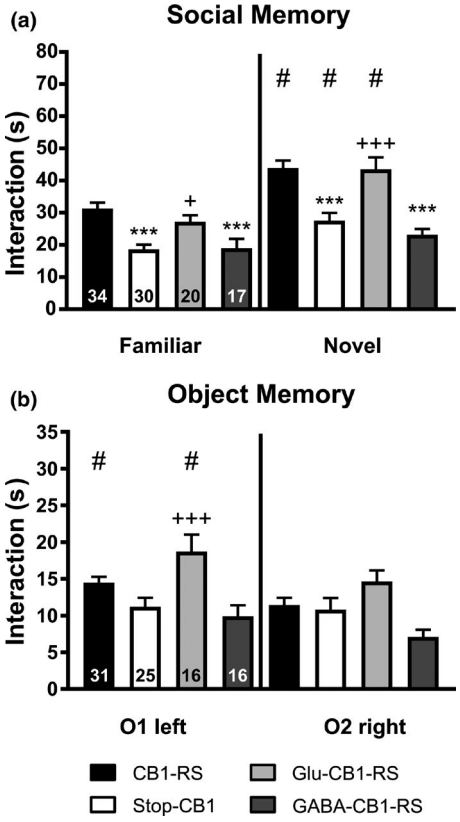

FIGURE 3 Effects of cell type-specific CB1 receptor rescue on memory for the first 5-min interval. Exploration of the familiar and the novel interaction partner during the social memory phase (a). Exploration of the familiar (O1) and the novel object (O2) of the object memory phase (b). Bars represent different genotypes. Graphs show mean values + *SEM*. Numbers displayed in the bars indicate the number of animals per genotype (*n*) used in each test. Univariate ANOVA $^{*}p < 0.05$, $^{**}p < 0.01$ (significantly different from CB1-RS); $^{+}p < 0.05$, $^{+++}p < 0.001$ (significantly different from Stop-CB1). $^{\#}$Animals display a significant preference for this interaction partner or object. O1, object 1; O2, object 2

0) for the interaction partner over the empty cage (Table S2; Figure 2a and S1a). However, the comparison of the DI amongst the different genotypes revealed no strong genotypic differences during the sociability test. The only exception was that Glu-CB1-RS and GABA-CB1-RS animals showed a small but significant increase in the DI when compared with Stop-CB1 animals (Table S3). In the social memory phase, all genotypes but the GABA-CB1-RS tended to favour the novel mouse over the familiar one (Table S2; Figure 3a and S3a). However, comparison of the DI amongst the different genotypes revealed no genotypic differences (Table S3).

3.2.2 | Object memory

In the object memory phase, the only significant difference was found between Stop-CB1 and Glu-CB1-RS animals during the first 5-min interval. Here, Glu-CB1-RS mice explored the familiar object significantly longer than Stop-CB1 animals (Table 5; Figure 3b and S3b). Univariate ANOVA evaluation of the first 5-min interval O2 right and the second 5-min interval O1 left reached significance due to differences between Glu-CB1-RS and GABA-CB1-RS only (Table 5; Figure 3b and S3b).

Over the 10 min of the object exploration phase, no side preference had been observed. Interestingly, in the object memory phase, all animals with the exception of the GABA-CB1-RS showed a clear preference for the familiar O1 (Table S2; Figure 3b and S3b). No genotype differences were found for the DI between objects, i.e., for "how strongly animals preferred one object to the other" (Table S3).

4 | DISCUSSION

Using novel transgenic mouse models, we were able to further underline the regulatory function of the CB1 receptor in forebrain GABAergic and dorsal telencephalic glutamatergic neurons. Consistent with previously published data (Häring et al., 2011; Lafenêtre et al., 2009; Rey et al., 2012; Terzian et al., 2014), we observed opposite behavioural outcomes regarding exploration depending on which CB1 receptor population is functional. Thus, these findings support the importance of the eCB system in cortical GABAergic and glutamatergic circuits to prevent neuronal and behavioural imbalance (Lutz et al., 2015; Ruehle et al., 2012).

4.1 | Impact of cell type-specific CB1 receptor signalling on locomotor behaviour

In our study, genotype differences in locomotion, which might confound exploration behaviour, were negligible and hence can be ignored. The only significant difference was observed between Glu-CB1-RS and Stop-CB1 mice versus CB1-RS animals in the initial minutes of the open field test. In this test, Glu-CB1-RS and Stop-CB1 mice showed an increased level of initial spontaneous locomotor activity, indicating a potential habituation/novelty phenotype rather than a locomotion phenotype (Table 2; Figure 1). Studies on conditional glutamatergic and GABAergic CB1 knock-out animals are consistent with our results, as none of them showed a robust locomotion phenotype in the mutant mice (Häring et al., 2011; Monory et al., 2007). Thus, basal locomotor activity does not seem to be influenced by endocannabinoid signalling at dorsal telencephalic glutamatergic or forebrain GABAergic CB1 receptors and accordingly is not expected to have interfered with exploration.

4.2 | Sufficiency of cell type-specific CB1 receptor signalling for social interaction

In our study, the ubiquitous lack of CB1 receptor expression due to the presence of a stop cassette upstream of the CB1 receptor coding sequence led to a strong decrease in social interaction but not social preference as compared with CB1-RS animals (Figure 2a and b, and S1a). Reactivation of the CB1 receptor expression in glutamatergic neurons rescued or even increased interaction compared with the CB1-RS phenotype. The reactivation in GABAergic neurons led to decreased interaction (Figures 2 and 3). Our findings might explain the contradictory results reported by Klugmann and colleagues (2011), who investigated rats overexpressing the CB1 receptor in the prefrontal cortex (PFC) (Klugmann et al., 2011). In their study, rats overexpressing the CB1 receptor in both glutamatergic and GABAergic neurons displayed an increase in social interaction with an unknown partner. However, these animals showed a significant decrease in overall physical social contact and even displayed increased evasion behaviour. Considering our own findings, the evasion behaviour and decrease in sociability might be based on the increased number of CB1 receptors on GABAergic interneurons. In contrast, the increased inhibitory effect on glutamatergic neurons projecting from the PFC might be responsible for the increase in active interaction behaviour. In particular, the modulation of glutamatergic projections to the amygdala might play an important role, as these projections have been shown earlier to be essential for social behaviour (Pape & Pare, 2010; Trezza et al., 2012). Thus, Trezza and colleagues demonstrated that the local administration of rimonabant (CB1 receptor antagonist) and URB597 (inhibitor of the endocannabinoid degrading enzyme fatty acid amide hydrolase, FAAH) into the amygdala reduced and induced play behaviour in rats, respectively. Additionally, recent studies using a viral approach to overexpress FAAH locally and thereby reduce

CB1 signalling through anandamide produced interesting insights. Rats with FAAH overexpression in the basolateral amygdala showed a reduction of fear and increased activity levels, which seemed to be based on increased GABAergic signalling (Morena et al., 2019), whereas overexpression in hippocampal glutamatergic neurons resulted in increased anxiety-like behaviour in mice (Zimmermann et al., 2019).

4.3 | Sufficiency of cell type-specific CB1 receptor signalling for exploratory behaviour

In contrast to social interaction, object exploratory behaviour was not significantly altered by the absence of CB1 signalling in the Stop-CB1 mice (Figure 2c and S1b). Therefore, the deficit in social investigatory drive most probably is not caused by a general decrease in sociability, exploration or activity behaviour. Instead, it seems to depend on increased anxiety levels, which were detected previously in these mice (Ruehle et al., 2013). Earlier studies on CB1 receptor knockout animals further support this conclusion. Thus, using pharmacological and/or genetic approaches, several studies showed that CB1 receptor deactivation increases anxiety behaviour, such as self-grooming and freezing, while mice also display less social and object exploration than wild-type controls (Helfand et al., 2017; Jacob et al., 2009; Litvin et al., 2013; Terzian et al., 2014). In line with our experiments, Jacob and co-workers did not find a significant difference in object investigation under low-aversive conditions (light intensity 30 lx). Furthermore, the deletion of the endocannabinoid degrading enzyme FAAH, resulting in increased anandamide levels, had the opposite effect, namely, an increase in the exploratory drive (Helfand et al., 2017). In a study on rats, a gain of function mutation in the CB1 receptor gene prolonged juvenile behaviour, including increased exploratory drive (Schneider et al., 2015).

In our experiments, Glu-CB1-RS mice either showed a partial or complete rescue of the animate exploratory phenotype observed in Stop-CB1 animals (Figure 2a and b, and S1a), and in the NORT they even reached inanimate exploration levels that were significantly higher than those measured in control (CB1-RS) animals. This finding is corroborated by conditional loss-of-function studies which demonstrated a decrease in social and object exploration in animals with a deletion of the CB1 receptor in dorsal telencephalic glutamatergic neurons under low light conditions (Häring et al., 2011; Jacob et al., 2009; Lafenêtre et al., 2009; Terzian et al., 2014). Therefore, it appears that CB1 receptor-mediated inhibition of dorsal telencephalic glutamate release promotes novelty-seeking behaviour, making this receptor subpopulation both necessary and sufficient for animate as well as inanimate exploration.

Reactivation of the CB1 receptor expression in GABAergic neurons, however, resulted in behavioural performances strongly dependent on the time interval analysed independent of the paradigm. Within the first 5 min, GABA-CB1-RS animals did not show a phenotype rescue regarding general exploration (Figure 2). They even explored significantly less than CB1-RS animals when Stop-CB1 mice did not show a difference (Figure 2a and c), whereas, in the second 5-min intervals (Figure S1), GABA-CB1-RS animals either displayed partial rescues or completely lost significant differences towards control animals. In agreement with our data, loss-of-function studies on mice with a conditional deletion of the CB1 receptor on GABAergic neurons have shown an increase in social and object exploration in these animals (Häring et al., 2011; Lafenêtre et al., 2009; Terzian et al., 2014). Lafenêtre and colleagues (2009) furthermore reported that the GABA-CB1-KO behaved in an object interaction task "as if they were already familiar" with the stimulus. Since CB1 receptor function in GABAergic neurons is suggested to be responsible for the anxiogenic response to high doses of cannabinoids (Rey et al., 2012), it likely explains the neophobic behaviour observed in our experiments in GABA-CB1-RS mice. Interestingly, a previous study of our group showed a significant rescue of the anxiety phenotype in the GABA-CB1-RS mice in the elevated plus-maze test (Remmers et al., 2017), suggesting distinct roles for the GABAergic CB1 receptor population depending on the situation. It is unlikely that a general sociability phenotype was the reason for their exploration deficit as GABA-CB1-RS showed a normal preference for animate over inanimate interaction (Table S2 and S3).

4.4 | Impact of cell type-specific CB1 receptor signalling on recognition memory

In general, no strong differences regarding the DI were observed between the genotypes in the sociability test and NORT, indicating that all groups formed a similarly strong memory (Table S3). Yet, only GABA-CB1-RS mice distinguished neither between the familiar and novel objects nor between the familiar and novel interaction partners (DI not significantly different from 0), suggesting a memory deficit in these mice. All other groups preferred the novel over the familiar interaction partner in the first 5-min interval of the social memory phase and, therefore, were considered as having an intact memory (Table S2).

Interestingly, in the object memory phase of the NORT, CB1-RS, Stop-CB1, and Glu-CB1-RS showed a clear preference for the familiar object over the novel one. Particularly, CB1-RS and Glu-CB1-RS displayed this behaviour in both 5-min intervals (Table S2). A preference for the familiar object is uncommon, but since object discrimination is still given, does not point to a deficit in memory performance. Similar findings have been reviewed by Ennaceur (2010). It might be argued that such an outcome may result when the degree of novelty of

the new object is too high. Therefore, the apprehension of an unfamiliar object could have outweighed the curiosity to investigate it and thus would have led to the avoidance of the novel object (Hughes, 1997; Montgomery, 1955; Russell, 1973).

4.5 | Confounding factors

It should be kept in mind that ubiquitous CB1 receptor deletion can induce developmental alterations (Galve-Roperh et al., 2013; Psychoyos et al., 2012) and even seems to promote an early decline of cognitive function (Bilkei-Gorzo et al., 2005). Hence, part of the behavioural differences observed in the present study might be caused by developmental effects. However, we did not find any differences between the discrimination indices of CB1-RS and Stop-CB1 animals, which argue against confounding cognitive impairments in our animals (Table S3). In addition, overall locomotion was, if at all, slightly increased rather than reduced (Figure 1) which makes locomotion deficits in the Stop-CB1 mice rather unlikely.

Additionally, we evaluated possible confounding by an innate preference for either O1 or O2. In a pre-test with CB1-RS mice, the mice were allowed to explore both objects simultaneously for 10 min during which no innate preference could be observed (Table S4). Furthermore, we compared the DI between genotypes for "climbing on objects." While all mice preferred to climb on object B, there was no genotype difference between them (Table S5 and S6).

5 | CONCLUSION

Our results underline the high relevance of the eCB function in the balance of behaviour. This study provides new insights into eCB function as our unique mouse models allow us to assess for the first time the potential sufficiency of distinct CB1 receptor populations to maintain wild-type-like behaviour. We have shown that this particularly holds true for the CB1 receptor expression in glutamatergic neurons. CB1 receptor signalling exclusively in GABAergic neurons proved not only to have the opposite effect on exploration but also seems to be involved in habituation processes and thus needs to be addressed further in future studies. Therefore, the regulatory properties of the eCB system on the cortical excitatory and inhibitory drive should also be addressed with respect to psychiatric disorders. In fact, a cortical control in neuronal pathologies caused by imbalanced GABAergic and glutamatergic transmission has already been suggested opening up a therapeutic avenue to pharmacologically restore a possible cortical imbalance in disorders such as autism or schizophrenia (Amitai et al., 2012; Del Arco et al., 2011; Chao et al., 2010; Guidali et al., 2011; Javitt, 2010; Sala et al., 2011).

Taken together, our rescue approach adds a crucial piece to the puzzle of the opposing roles of CB1 receptor signalling in cortical GABAergic and glutamatergic neurons on behavioural performance, in particular on exploration.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

AUTHORS' CONTRIBUTIONS

VDG, designed the study, performed experiments, analysed the data, drafted the paper, edited the paper; SR, designed the study, contributed resources, edited the paper; BL, designed the study, acquired funding, edited the paper; MH, designed the study, performed experiments, drafted the paper, edited the paper; FR, designed the study, analysed the data, drafted the paper, edited the paper.

DATA AVAILABILITY STATEMENT

Upon request, an FRT link will be provided.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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