

How much fear is in anxiety?

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8 **Abstract** The selective breeding for extreme behavior on the elevated plus-maze (EPM) resulted
9 in two mouse lines namely high-anxiety behaving (HAB) and low-anxiety behaving (LAB) mice.
10 Using novel behavioral tests we demonstrate that HAB animals additionally exhibit maladaptive
11 escape behavior and defensive vocalizations, whereas LAB mice show profound deficits in escaping
12 from approaching threats which partially results from sensory deficits. We could relate these
13 behavioral distortions to tonic changes in brain activity within the periaqueductal gray (PAG) in HAB
14 mice and the superior colliculus (SC) in LAB mice, using in vivo manganese-enhanced MRI (MEMRI)
15 followed by pharmacological or chemogenetic interventions. Therefore, midbrain-tectal structures
16 govern the expression of both anxiety-like behavior and defensive responses. Our results challenge
17 the uncritical use of the anthropomorphic terms *anxiety* or *anxiety-like* for the description of mouse
18 behavior, as they imply higher cognitive processes, which are not necessarily in place.

20 Introduction

21 The anthropomorphic terms *anxiety* or *anxiety-like* are widely used for the description of affective
22 states in laboratory animals. The definition for anxiety (*American Psychiatric Association, 2013*)
23 includes worries about distant or potential threats while the occurrence of exaggerated anxiety in
24 combination with constant ruminations about illusionary threats indicates an anxiety disorder.
25 *Fear* on the other hand describes the affective state (*'being afraid'*) which is elicited with respect to
26 an explicit, threatening stimulus.

27 The behavioral repertoire of fear - i.e. the sum of defensive responses - results from a recruit-
28 ment of the *defensive survival circuits* (*LeDoux, 2014*). Its functions are either increasing the distance
29 between the subject and the threat (flight), rendering the subject invisible to the threat (freezing)
30 or ultimately enabling the subject to fight. This includes the autonomic and neuroendocrine
31 processes which prepare the creature for a successful flight e.g. reflected by increased heart and
32 respiratory rate and release of stress hormones via increased hypothalamus-pituitary-adrenal-
33 medulla (HPA) axis activity. As previously suggested, this condition is described best as the *defensive*
34 *organismic state* (*LeDoux, 2014*). Therefore, it is just to say that the subjective feeling of being
35 anxious or afraid are cognitive processes, while the behavioral expression of anxiety, fear and panic
36 are physical or bodily processes which are typically orchestrated by subcortical and mesencephalic
37 structures (*LeDoux and Pine, 2016*). In laboratory animals, like mice and rats, we lack the access to
38 these subjective inner cognitive states, but have to solely rely on the interpretation of physiological
39 and behavioral data.

40 A variety of behavioral testing paradigms therefore aims to assess states of *anxiety*, *fear* or *panic*
41 based on the type and quality of evoked defensive behaviors in response to specific stimuli or
42 contexts (*for review see Cryan and Holmes, 2005; Calhoun and Tye, 2015*). Hereby, more subtle be-

43 haviors like avoiding exposed and brightly illuminated areas on an elevated plus maze (EPM)(*Pellow*
44 *et al., 1985*) are interpreted as *anxiety*. In contrast, the sudden jumping (a flight reaction completely
45 different from startle response) followed by pronounced immobility (freezing) upon the onset
46 of a previously negatively conditioned tone (auditory/Pavlovian fear conditioning; *for review see*
47 *Maren, 2001*) is commonly associated with *fear*. These tests suggest a sharp distinction between
48 the behavioral measures of *anxiety* and *fear*. For instance, auditory fear conditioning experiments
49 paved the way for an in depth understanding of the amygdalar circuits underlying the expression a
50 single characteristic defensive response (i.e., freezing) (*for review see Tovote et al., 2015*). In more
51 complex and ethological relevant testing situations, however, one can observe a gradual transition
52 from risk assessment to avoidance and flight or tonic immobility and ultimately fight/panic-like
53 jumping as a function of the threat's imminence (i.e. defensive distance) and the ability to escape
54 (*Ratner, 1967, 1975; Blanchard et al., 1986; Blanchard and Blanchard, 1990; Blanchard et al., 1990,*
55 *1997, 2003*). This relationship was initially conceptualized as the *predatory imminence continuum*
56 (*Fanselow and Lester, 1988*) and later has been integrated into the *two-dimensional defense system*
57 (*McNaughton and Corr, 2004*). The two-dimensional defense system is of particular significance
58 as it comprehensively describes the interplay of *defensive avoidance* and *defensive approach* with
59 respect to the *defensive distance* (perceived distance to threat). In addition, it highlights the func-
60 tional hierarchy of dominant brain structures in the orchestration of the behavioral expression
61 of anxiety, fear and panic. In this context, McNaughton & Corr reappraise the function of the
62 periaqueductal gray (PAG) '*in the lowest levels of control of anxiety*' (*McNaughton and Corr, 2004*) (*see*
63 *also (McNaughton and Corr, 2018)*).

64
65 In this line of thinking we were interested to which extent the behavioral phenotype of a mouse
66 model for extremes in *trait anxiety* (1) is accompanied by altered levels of defensive responses,
67 and in addition (2) can be explained by changed neuronal activity in midbrain structures. As a
68 model organism we chose two mouse lines which were previously established from CD1 mice as
69 the result of a selective breeding approach based on the behavior on the EPM - a classical anxiety
70 test. Thereby hyperanxious high-anxiety behaving (HAB) and hypoanxious low-anxiety behaving
71 (LAB) mice were generated (*Krömer et al., 2005*) which are compared to normal-anxiety behaving
72 (NAB) mice. Besides the already mentioned *anxiety-like* phenotype on the EPM (*Krömer et al., 2005;*
73 *Bunck et al., 2009; Erhardt et al., 2011; Avrabos et al., 2013; Yen et al., 2013; Füchsl et al., 2014*),
74 these lines show also marked differences in other behavioral and physiological measures (*see*
75 *Table 1*). In HAB mice, most of the behavioral measures are biased towards immobility or lack of
76 exploratory drive. This bears the risk of false interpretations, since altered locomotor activity and/or
77 motivation might explain the extreme phenotypes as well. In the present study we comprehensively
78 re-characterize HAB, NAB and LAB (HNL) mice for their extreme behavioral phenotypes on the EPM.
79 We provide evidence that in HAB animals only ethobehavioral EPM measures and the levels of
80 autonomic arousal are sensitive to anxiolytic treatment. In addition, we demonstrate for the first
81 time that adult HAB animals show a disposition for sonic/audible vocalizations which is decreased
82 by the anxiolytic diazepam. Further, we show that the extremes in high or low *anxiety-like* behavior
83 of HAB and LAB animals are accompanied by paralleled alterations active in defensive responses
84 using two novel, multi-sensory tasks (Robocat and IndyMaze) which assay repeated, innate escape
85 behavior towards an approaching threatening stimulus. Hereby, we demonstrate that HAB animals
86 present maladaptively altered levels of defensive responses, while LAB animals exhibit a strongly
87 deficient reaction towards the threatening stimulus. Using several complementary strategies to
88 probe the visual capabilities of HNL animals (optomotor response, electroretinography, etc.), we
89 show that LAB animals suffer from complete retinal blindness. In order to assess tonic/basal in-vivo
90 whole-brain neuronal activity alterations in HAB and LAB animals, we employ manganese-enhanced
91 magnetic resonance imaging (MEMRI) (*Grünecker et al., 2010; Bedenk et al., 2018*). Thereby, we
92 provide evidence that HAB mice exhibit an increased neuronal activity within the PAG, while LAB
93 mice show a decreased activity in the deep layers of the superior colliculus (SC). Finally, using a

94 designer receptor exclusively activated by designer drugs (DREADD) approach in LAB mice or by
 95 applying localized injections of muscimol in HAB mice we are able to partially revert the extreme
 96 phenotypes in *anxiety-like* behavior in LAB and HAB animals.

Table 1. Physiological & Behavioral Phenotypes of HAB and LAB mice

Modality	Test	Measure/Param.	HAB	LAB	References
Anxiety	EPM	open-arm time	--	++	(Krömer et al., 2005; Bunck et al., 2009; Erhardt et al., 2011); (Avrabet et al., 2013; Yen et al., 2013; Füchsl et al., 2014)
	EPM	open-arm latency	++	•	(Krömer et al., 2005)
	DLB	time in light comp.	•	+	(Krömer et al., 2005)
	USV	no. of vocalizations	++	--	(Krömer et al., 2005)
	IA	step-down latency	++	n.a.	(Yen et al., 2012)
Fear	TMT	odor avoidance	+	•	(Sotnikov et al., 2011)
	FC	contextual, freezing	++	--	(Sartori et al., 2011a; Yen et al., 2012)
	FC	cued, freezing	++	--	(Sartori et al., 2011a; Yen et al., 2012)
	TM	FC, HR during CS	++	n.a.	(Gaburro et al., 2011)
	TM	FC, HRV during CS	-	n.a.	(Gaburro et al., 2011)
	ASR	105-115 dB	-	++	(Yen et al., 2012, 2013)
Locomotion	DLB	line crossings	--	++	(Krömer et al., 2005)
	DLB	rearing	--	++	(Krömer et al., 2005; Yen et al., 2013)
	HB	rearing	-	++	(Yen et al., 2013)
	OBS	homecage activity	•	+	(Krömer et al., 2005)
	TM	homecage activity	•	n.a.	(Gaburro et al., 2011)
	OF	distance	•	++	(Yen et al., 2013)
	OF	mobility time	--	++	(Yen et al., 2013)
Stress Reactivity	TMT	CORT release	•	•	(Sotnikov et al., 2011)
	FST	CORT release	--	•	(Sotnikov et al., 2014)
	DEX	CORT release	--	•	(Sotnikov et al., 2014)
Depression	TST	immobility	•/+	--	(Krömer et al., 2005; Bunck et al., 2009; Yen et al., 2013)
	FST	immobility	•/++	--	(Krömer et al., 2005; Bunck et al., 2009; Sah et al., 2012); (Sotnikov et al., 2014; Schmuckermair et al., 2013)
	SP	sucrose intake	--	n.a.	(Sah et al., 2012)
Addiction	CPP	cocaine-induced	+	n.a.	(Prast et al., 2014)
Spatial Navigation	WCM	re-learning	•	--	(Yen et al., 2013)
Physiology		fluid intake	n.a.	++	(Kessler et al., 2007)
		urine osmolarity	n.a.	--	(Kessler et al., 2007)
	IHC	GAD65/67 in amygdala	++	n.a.	(Tasan et al., 2011)
	VSDI	intra-amygdalar signal prop.	++	-	(Avrabet et al., 2013)

ASR acoustic startle response, CS conditioned stimulus, CORT corticosterone, CPP conditioned place preference, CRH corticotropin releasing hormone, DEX dexamethasone-suppression/CRH-stimulation test, DLB dark-light box, EPM elevated plus maze, FC auditory/contextual fear conditioning, FST forced swim test, HB holeboard test, HR heart rate, HRV heart rate variability, IA inhibitory avoidance, IHC immuno-histochemistry, OBS observation or visual scoring by experienced experimenter, OF open field, SP sucrose preference test, TMT 2,5-dihydro-2,4,5-trimethylthiazoline, TM telemetry, USV ultrasonic vocalizations, VSDI voltage-sensitive dye imaging, WCM water cross-maze. -- strong decrease; - slight decrease; • no change; + slight increase; ++ strong increase; n.a. not applicable.

Note: Only those references were taken into account which directly compare HAB to NAB and LAB to NAB.

97 Results

98 Behavioral Assessment of HAB, NAB, LAB mice on the Elevated Plus Maze

99 The elevated-plus maze (EPM) is considered to be a robust assay for the detection of altered *anxiety-*
 100 *like* behavior in mice. However, the standard test duration rarely exceeds 5-10 minutes (Komada
 101 et al., 2008), whereby strong inter-individual differences in avoidance behavior and especially their
 102 pharmacological modulation, are masked due to stringent cut-off criteria. In order to overcome
 103 this issue, we have extended the testing duration to 30 minutes and re-evaluated the behavior of
 104 HAB (N=11), NAB (N=7) and LAB (N=7) mice on the EPM, while focusing on the initial 5 minutes for
 105 all parameters, except for latency (0-30 min) and stretch-attend postures (0-15 min), to provide
 106 measures which are largely comparable to previous studies (see Fig 1A). Analysis of data obtained
 107 during the entire observation period revealed essentially the same findings (not shown).

108 Using this approach, significant group differences ($F_{2,22}=15.07$, $p<0.0001$) in the latencies to
 109 explore the open arms were revealed (Fig 1A). More than 45% of all HAB animals did not enter
 110 the open arm, even within the extended testing duration of 30 minutes compared to 0% in NAB

111 mice ($\chi^2=4.41$, $p=0.0358$). On the contrary all LAB animals explored the open arm with latencies
112 < 6 minutes. These distinct behavioral traits were also reflected by the percentage of time the
113 animals spent on the open arm: LAB animals $53.6\pm 11.3\%$ compared to $2.4\pm 0.8\%$ NAB ($F_{2,22}=26.25$,
114 $p<0.0001$). Additionally, LAB animals showed an overall increase in locomotor activity (1400.0 ± 171.7
115 cm vs. 723.0 ± 60.8 cm, $F_{2,22}=22.49$, $p<0.0001$). On the contrary, HAB animals spent more than 85%
116 of the time in the closed arm ($F_{2,22}=28.98$, $p<0.0001$), as they also avoided staying in the central
117 zone ($13.0\pm 2.3\%$ vs. $33.8\pm 4.0\%$, $F_{2,22}=12.96$, $p=0.002$). These observations are consistent with
118 previous reports of HAB, NAB and LAB behavior on the EPM (*Krömer et al., 2005; Bunck et al.,*
119 *2009; Erhardt et al., 2011; Avrabos et al., 2013; Yen et al., 2013; Füchsl et al., 2014*). The rather low
120 open-arm time shown by NAB mice may relate to the specific test conditions (we placed the EPM in
121 middle of a large, dimly lit room without additional surrounding enclosures). To complement the
122 traditional EPM parameters, the display of stretched-attend postures (SAP) (*Grant and Mackintosh,*
123 *1963*), a form of active risk assessment behavior, was analyzed as an ethobehavioral measure
124 (Fig 1B). It was previously shown that the number of SAPs decreases upon anxiolytic treatment
125 (*Kaesermann, 1986*) and increases with the anxiogenic 5-HT_{2C/1B} receptor antagonist mCPP (*Grewal*
126 *et al., 1997*). Moreover, the display of SAPs depend on the presence of an imminent threat or a
127 potential threatening situation and demonstrate the general motivation of the animals to explore
128 a potentially threatening environment (*Pinel et al., 1989*). LAB ($N=6$, one animal was excluded as
129 no SAPs were displayed) animals showed a significantly lower number of SAPs (Fig 1B; $F_{2,19}=29.84$,
130 $p<0.0001$; LAB 20.7 ± 5.9 vs. NAB 63.0 ± 4.3), whereas HAB animals were indistinguishable from NAB
131 (Fig 1B; $N=5$, two animal were excluded as no SAPs were displayed). Looking at the overall duration
132 of displayed SAPs, HAB animals showed increased measures (HAB 222.4 ± 16.8 s vs. NAB 158.0 ± 15.2
133 s), whereas LAB animals spent on average only 33.7 ± 12.3 seconds displaying SAPs ($F_{2,19}=32.74$,
134 $p<0.0001$). If analyzed in 5 min bins, NAB animals could adapt to the EPM and the duration of
135 displayed SAPs decayed. On the contrary, HAB animals showed an elevated non-decaying response
136 after 15 minutes (group \times time interaction: $F_{2,28}=3.587$, $p=0.0410$; 2-way rmANOVA) and higher
137 autonomic arousal, which was reflected by significantly increased defecation (*Hall, 1934*) during the
138 EPM task (Fig 1C; 11.6 ± 1.2 vs. 7.7 ± 0.7 , $F_{2,21}=4.779$, $p<0.0195$).

139

140 Before the animals were placed on the EPM, every subject was tested for the disposition to
141 emit sonic vocalizations by lifting them 3 times from grid cage top (*Whitney, 1970*). Animals which
142 vocalized at least once, were counted as ‘vocalizers’. Whereas none of the NAB ($N=13$) or LAB
143 ($N=15$) animals emitted even a single call, 47% of HAB ($N=15$) animals vocalized at least once (Fig 1D;
144 $\chi^2=15.61$, $p=0.0004$).

145
146 In order to investigate to which extent the phenotype of HAB mice can be modulated with
147 traditional anxiolytics, we injected diazepam (DZP, 1 mg/kg i.p.) or vehicle (saline) to separate
148 groups of experimentally naive HAB mice ($N=13$, each). None of the classical EPM parameters
149 were sensitive to DZP treatment, except for an increase in locomotor activity (Fig 1E; $t_{24}=2.174$,
150 $p=0.0398$). Neither the total number nor the total duration of SAPs were significantly altered by
151 DZP (Fig 1F). However, analysis in 5-min bins revealed that DZP turned the non-decaying display
152 of SAPs shown by vehicle-treated HAB into a decaying trajectory (treatment \times time: interaction:
153 $F_{2,28}=3.587$, $p=0.0410$; 2-way rmANOVA) which resembles the situation in NAB mice. In addition,
154 DZP treatment decreased defecation (Fig 1G; 1.5 ± 0.5 vs. 5.8 ± 1.2 , $t_{24}=3.344$, $p=0.0027$) and the
155 disposition to vocalize during a 5 minute tail-suspension test (TST) (Fig 1H; 3 out of 13 vs. 9 out of 13,
156 two-sided Fisher’s exact test $p=0.0472$). The higher absolute incidence of vocalizers, compared to
157 the data shown in Fig 1D, is most likely due to prior injection stress. The lower absolute defecation
158 scores, in turn, might be partially ascribed to defecation during the injection procedure. Taken
159 together, HAB, NAB and LAB animals show a robust behavioral phenotype on the EPM. Further,
160 under our experimental conditions, the traditional EPM measures are not sensitive to diazepam-
161 treatment, but more ethologically relevant measures like autonomic arousal, vocalization and active
162 risk assessment.

163 **Two Novel Ethologically Inspired Testing Situations Reveals Extremes in Innate De-** 164 **fensive Responses in HAB and LAB Mice**

165 The behavioral measures obtained on the EPM are indicative of an approach-avoidance situation
166 which became manifest differently in HAB and LAB mice. The term *anxiety* test for the EPM infers
167 an inner conflict which misleadingly points towards higher cognitive processes, mediated for
168 example by the prefrontal areas. Looking at avoidance behavior separately, it becomes obvious that
169 there is a strong subcortical component which is in a continuum to flight and *panic-like* reactions,
170 involving most likely the amygdala, ventromedial hypothalamus, periaqueductal gray and the
171 superior colliculus (*McNaughton and Corr, 2018*). Therefore we were interested if the altered EPM
172 phenotype of HAB and LAB is accompanied by changes in defensive responses as it has been
173 suggested previously to be the case with conditioned fear (*Sartori et al., 2011b; Yen et al., 2012*). In
174 order to circumvent learning mediated effects, we focused on innate defensive responses upon
175 acute confrontation with a (potential) threat.

176 Paradigms which assess general innate *fear* levels should incorporate multi-sensory stimuli
177 and allow for repeated testing and temporally confined exposure. In lack of appropriate testing
178 situations, we have developed two novel paradigms: the Robocat (*see*), which is based on a
179 previously published design by *Choi and Kim (2010)*, and the IndyMaze, which is inspired by a
180 popular movie (*Spielberg and Marshall, 1981*) (For a detailed description of both tests *see* section
181 *Methods and Materials*). The different behavioral readouts obtained in the Robocat task are depicted
182 in Fig 2A. The mouse could either activate the Robocat and subsequently display a flight response,
183 activate the Robocat but simply bypassing it or activate the Robocat and collide with it. The innate
184 defensive responses of HAB ($N=7$), NAB ($N=6$) and LAB ($N=9$) mice were assessed using the Robocat
185 task. Fig 2B depicts the percentage of animals which displayed the respective behaviors at least
186 once during a 10 minute exposure to the Robocat. During this trial the animals activated the
187 Robocat several times (HAB 2.4 ± 0.4 , NAB 3.5 ± 0.6 , LAB 10.8 ± 2.1). HAB animals were not able to
188 adapt to the Robocat’s activation and showed a flight response at all encounters (Fisher’s exact
189 $p=0.021$), they never bypassed (Fisher’s exact $p=0.0047$) nor collided with it. On the contrary NAB

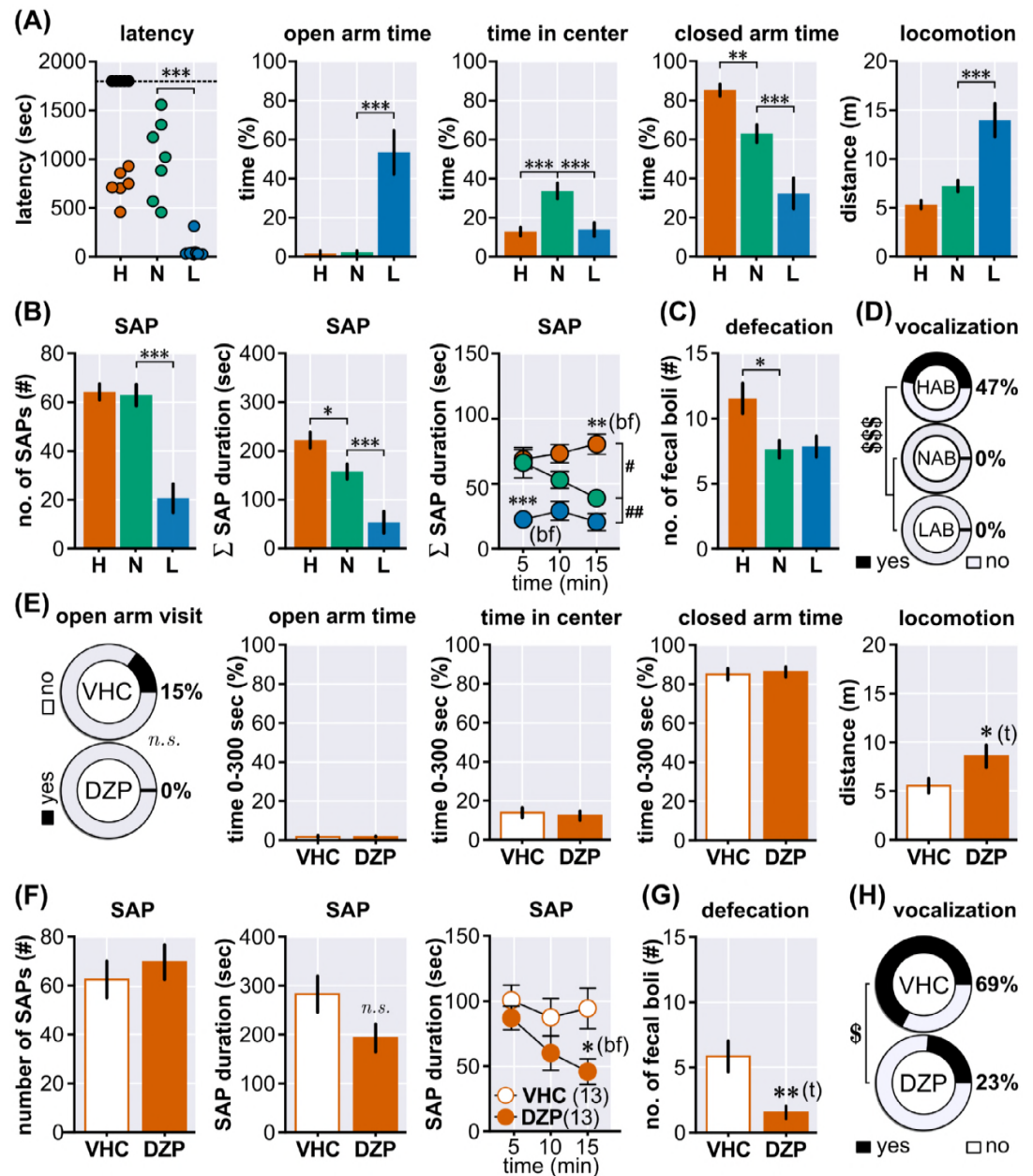


Figure 1. Behavioral Assessment of HAB, NAB, LAB Mice on the EPM

(A) Using the standard EPM but with an extended cut-off time of 30 min the following behavioral parameters were assessed for HAB ($N=11$), NAB ($N=7$) and LAB ($N=7$) animals: the latency to enter the open arm, open-arm time (first 5 minutes; 0-5 min), central zone time (0-5 min), closed arm time (0-5 min) and the distance the animals have traveled (0-5 min). (B) In addition to the classical EPM parameters we have also investigated the display of stretched-attend postures (SAP) which serves as a measure of active risk assessment: the total number of SAPs during the first 15 min of the task, the total duration of SAPs during the first 15 min, and the duration of SAPs in 5 minute bins. (C) Defecation during EPM exposure (number of fecal boli) as an indirect measure of autonomic arousal. (D) Disposition to emit sonic/audible vocalizations. (E) A new cohort of experimentally naive HAB mice was treated with diazepam (1 mg/kg, i.p.; $N=13$) or vehicle (saline, $N=13$) before exposure to the EPM. (F) Stretched-attend posture display of HAB animals during EPM with diazepam/vehicle treatment. (G) Defecation of HAB mice. (H) In order to assess the disposition to vocalize in standardized manner, the diazepam/vehicle treated HAB animals were subjected to a 5 min tail-suspension test, while audio signal were recorded and scored offline. Asterisks indicate significance values obtained by t -tests (t) or 1-way ANOVA followed by Newman-Keuls Multiple Comparison/Bonferroni post-hoc (bf) tests, * $p<0.05$, ** $p<0.01$, *** $p<0.001$; dollar signs indicate significance values obtained by χ^2 tests, \$ $p<0.05$, \$\$\$ $p<0.001$; hashes indicate group effects obtained by 2-way ANOVA, ## $p<0.01$. Values are given as mean \pm SEM.

190 animals, displayed a well-balanced behavioral profile: the minority of all animals fled the Robocat
 191 (33%) or got hit by the it (17%), while 83% of all NAB mice tolerated and bypassed the threatening
 192 stimulus at least once. This is contrasted by the behavior of LAB mice: no single animal fled upon
 193 the Robocat's movement, but all bypassed it. Most strikingly however, the vast majority of LAB mice

194 89% even collided with it at least once (Fisher's exact $p=0.011$).
195 The Robocat task revealed differential defensive responses between HAB and NAB, whereby the
196 inability of HAB mice to bypass the Robocat can be interpreted as maladaptive behavior. At the
197 same time the high degree of controllability (allowing a bypass or withdrawal from the arena to
198 avoid activation/confrontation) does not allow to ask whether NAB and LAB show different levels of
199 defensive responses: the inability to express defensive responses, and a high degree of adaptation
200 would result both in a decreased level of observable defensive reactions. In order to avoid this
201 confounding variable we have developed the IndyMaze. In this test an animal is confronted with a
202 rolling (25 cm/s) styrofoam ball (100 g) in a tilted ($<1^\circ$) and narrow tunnel. Therefore, every trial
203 involves a direct encounter with the threatening stimulus. The operational procedure is depicted in
204 Fig 2C. First, the animals are free to enter the arena, which gives the latency to first exit, a measure
205 comparable to other emergence tasks (Fig 2D). This measure corresponds to the exit latency on
206 the EPM. HAB animals showed high latencies to exit the home compartment (HAB 977.4 ± 79.2 s vs.
207 NAB 392.1 ± 66.1 s) whereas LAB animals were not different from NAB ($F_{2,50}=12.64$, $p<0.0001$). A
208 significant amount of HAB animals never left the home compartment (Fig 2E) within 30 minutes
209 ($\chi^2_{2,N=62}=6.671$, $p=0.0356$). Once the animals have left the home compartment, they explored the
210 entire arena (Fig 2F) with equally low latency (HAB 68.8 ± 10.1 s; NAB 213.1 ± 72.8 s; LAB 100.4 ± 18.2
211 s). This demonstrates comparable levels of exploratory drive in all three lines and precludes that
212 the increased latency to the 1st exit simply results from a lack of motivation or impaired locomotor
213 behavior. Looking at the defensive responses (Fig 2G), which included preemptive flight responses
214 or a retrieval after the ball has hit the animals, it is evident that both HAB and NAB are able to
215 respond appropriately towards approaching threatening stimuli, whereas 60% of LAB animals
216 exhibited significant deficits and failed to generate at least one defensive reaction ($\chi^2_{1,N=27}=13.11$,
217 $p=0.0014$). In order to test whether the behavioral readouts obtained using the IndyMaze can be
218 modulated with anxiolytics, another cohort of HAB animals was treated with diazepam (DZP, 1
219 mg/kg, $N=13$) or vehicle (VHC, saline, $N=12$) and were subjected to the IndyMaze task. The DZP
220 treatment could significantly decrease the latency to 1st exit (Fig 2H; VHC 1011.0 ± 153.4 s vs. DZP
221 595.5 ± 133.5 s; Mann-Whitney, two-tailed, $U_{n1=208, n2=117}=39.00$, $p<0.0363$), indicative of an anxiolytic
222 effect, while leaving latency for end-exploration unaffected (*see*). However, DZP treatment was
223 ineffective in modulating defensive responses (defensive responsivity: VHC 100%, DZP 100%). NAB
224 and HAB, but not LAB, mice showed short-term avoidance of additional encounters with the ball, as
225 indicated by the increase in latency until re-entering the middle part of the arena. One week later,
226 both HAB and NAB mice showed a highly significant decrease in latency to 1st entry compared
227 to the first exposure. Nevertheless, only NAB mice showed long-term avoidance of the middle
228 segment of the arena, which is indicative of maladaptive consequences of heightened fear/ anxiety
229 for the development of avoidance behavior (data not shown).

230 In summary, both tasks, the Robocat and IndyMaze, have proven to be valid tools to assay innate
231 defensive responses in mice. In addition, the IndyMaze task permits also the parallel assessment
232 of inhibitory avoidance behavior. Using both tasks, we could demonstrate that HAB mice show
233 maladaptive levels of defensive responses. LAB animals, in contrast, exhibited strong deficits to
234 escape imminent threats.

235 **Complete Retinal Blindness in LAB Mice**

236 The remarkable ignorance of LAB mice to approaching objects forced us to look for differences
237 in visual perception. A standard test for visual acuity in mice is the assessment of the optomotor
238 response (OMR) (*Thaung et al., 2002; Abdeljalil et al., 2005*). This test is based on the tracking
239 behavior of mice in response to horizontally moving stripes. For this test, mice are placed on a fixed
240 platform within a rotating cylinder lined with stripes of different width to probe visual acuity (Fig 3A
241 *inset*). We modified this testing procedure in order to fit to all five mouse lines (B6, CD1, HAB, NAB
242 and LAB) in a way that we have used only one, relatively large grating (0.5 cycles/degree) and in
243 addition scored every head movement if it was concordant with the cylinders rotational direction.

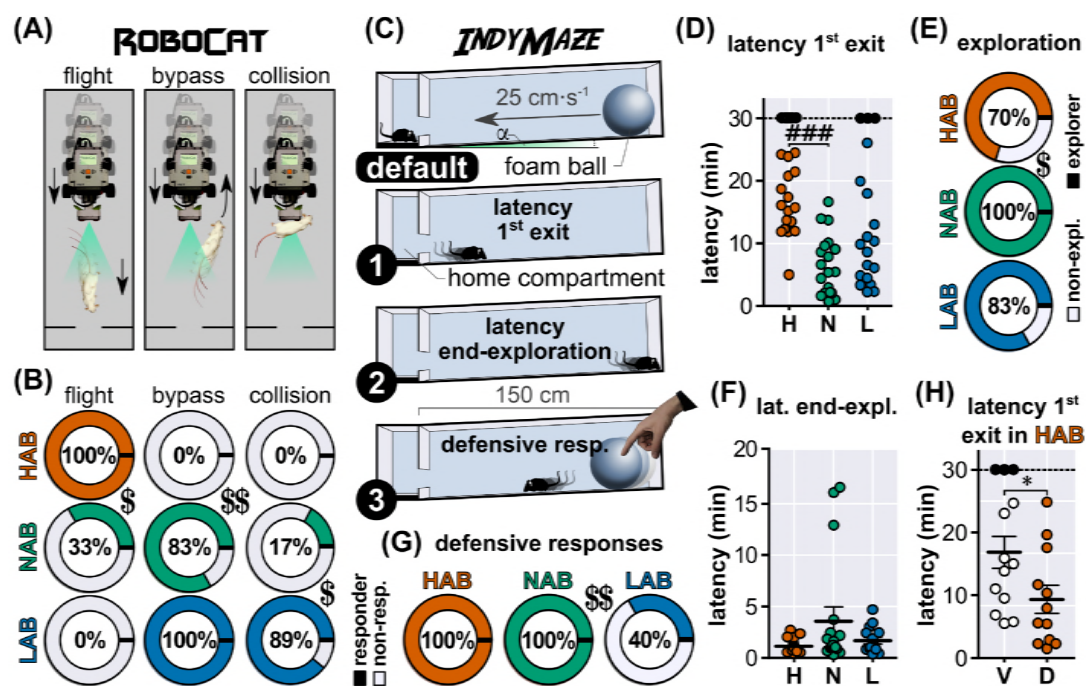


Figure 2. Two Novel Ethologically Inspired Testing Situations Reveals Extremes in Innate Defensive Responses in HAB and LAB Mice

A) The three different behavioral measures obtained in the Robocat task, whose appearance have been scored: flight, bypass and collision. **(B)** Using the Robocat, we have investigated the fear responses of HAB ($N=7$, red), NAB ($N=6$, green) and LAB ($N=9$, blue) animals (analyzed using Fisher's exact tests). Values are percentages of animals which showed the respective behavior at least once. **(C)** Schematic description of the IndyMaze (default) operational procedure as well as the different behavioral measures (latency to 1st exit I, latency for end-exploration II and flight response III). Using the IndyMaze we have tested different cohorts of HAB ($N=24$), NAB ($N=19$) and LAB ($N=20$) animals. **(D)** Quantification of the latency to 1st; black-filled circles indicate animals which did not leave the start arm, HAB ($N=7$), NAB ($N=0$) and LAB ($N=4$), those animals were excluded from the 1-way ANOVA. **(E)** Quantification of the number of animals which explored the arena. **(F)** Quantification of the latency for end-exploration excluding animals which did not enter the arena at all, as shown in *D*. Note: If the animals left the start compartment, they all explored the arena to its end with comparable vigor. **(G)** Quantification of the occurrence of fear responses at least once during 3 encounters of the approaching styrofoam ball (this includes preemptive fear responses, as well as fear responses after the ball had hit the animal). **(H)** Another cohort of HAB animals was treated with diazepam (DZP, 1 mg/kg, i.p.) ($N=13$) or vehicle (VHC, saline, $N=12$) and subjected to the IndyMaze, and the latency to 1st exit was quantified. Asterisks indicate significance values obtained by Mann-Whitney test, * = $p < 0.05$; dollar signs indicate significance values obtained by χ^2 or Fisher's exact tests, \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$; hashes indicate significance values obtained by 1-way ANOVA followed by Newman-Keuls Multiple Comparison test, ### $p < 0.001$. Values are given as mean \pm SEM.

244 Therefore we have used this test to assess vision in general, rather than visual acuity. Using this
 245 approach, we could observe significant strain differences (Fig 3A; $F_{4,54}=93.13$, $p < 0.0001$). B6 mice
 246 outperformed all other strains by far (B6 16.9 ± 0.9 OMR/min), whereas among the albino animals
 247 HAB animals showed the strongest responses (5.5 ± 0.8 OMR/min). Both, CD1 (2.6 ± 0.7 OMR/min)
 248 and NAB (2.4 ± 0.5 OMR/min) animals responded similar, but LAB animals failed to show any clear
 249 optomotor responses (0.2 ± 0.1 OMR/min).

250 As LAB mice also have been reported to exhibit certain phenomenological similarities to ADHD
 251 (Yen *et al.*, 2013), we cannot exclude the possibility that these animals perceive but are unable
 252 to attend to the visual stimuli and thus fail to show an appropriate response. Therefore, the
 253 retinal function of all five mouse strains was investigated using flash electroretinography (fERG)
 254 measurements in the anesthetized animal. The fERG setup (depicted in Fig 3B top) consisted of
 255 a differential amplifier usually used for in-vivo extracellular neural recordings (Siegle *et al.*, 2017),
 256 whereby the reference electrode was placed on the shaded eye. The other eye was stimulated
 257 with a custom built miniature eyecup, equipped with a white LED, in combination with a custom
 258 built LED driver. This setup allowed the reliable detection of electroretinographic signals and
 259 the dissection of the b-wave component of fERG (Fig 3B bottom). The fERGs acquired in scotopic
 260 (dark-adapted for >3h) as well under photopic conditions at three different light flash intensities
 261 (0.23 , 128 and 1.69 log photoisomerizations \times rod $^{-1}$ \times s $^{-1}$), showed strong deflections for B6, CD1,
 262 HAB and NAB ($N=6$, each) animals (Fig 3C). However, in LAB animals ($N=6$) there was no detectable

263 electrophysiological response (scotopic: Group $F_{4,25}=14.38$, $p<0.0001$, Fig 3D; photopic: Group
 264 $F_{4,25}=8.77$, $p=0.0001$, 2-way rmANOVA; Fig 3E). To further determine the cause for the absence
 265 of electroretinographic responses, a histological analysis of retinal sections of all strains ($N=3$,
 266 each, right eye) was conducted and an absence of the outer nuclear layer (ONL) and the subjacent
 267 inner/outer segments (IS/OS) was observed in LAB animals (Fig 3F). As the founder strain for LAB
 268 animals (CD1) is known to exhibit incidences of a recessive *rd1* retinal degeneration (*Serfilippi*
 269 *et al.*, 2004), we employed a polymerase chain reaction (PCR) genotyping screening for all strains
 270 ($N=4$, each, tail biopsy) (*Chang et al.*, 2013). The test (Fig 3G) revealed that LAB animals exhibit
 271 a homozygous mutation in the *Pde6b*^{rd1+/+} allele which is indicative of the retinal degeneration 1
 272 mutation which leads to blindness shortly after birth. Therefore, it is to conclude that LAB animals
 273 (tested at an age of 3-6 month of age) suffer from complete retinal blindness, which is the reason
 274 for the inability to escape approaching threatening stimuli, like the Robocat (Fig 2B). But blindness
 275 does not explain why still only 40% of LAB animals showed a flight response even after hit by the
 276 ball in the IndyMaze task (Fig 2G).

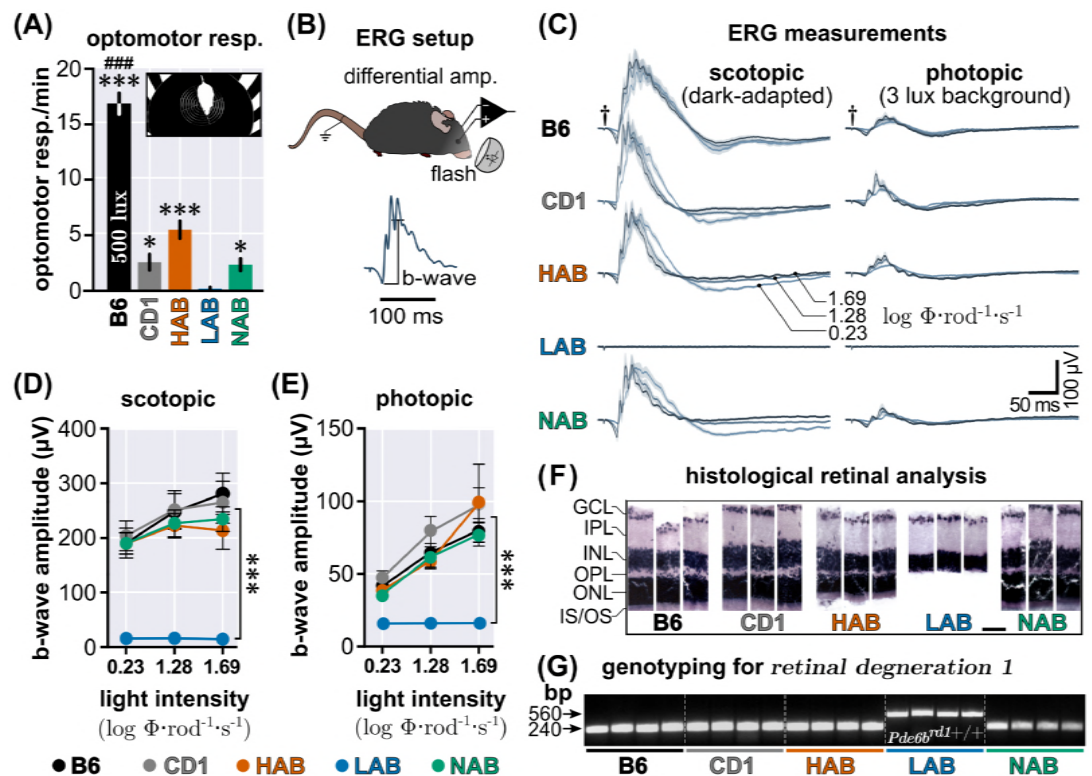


Figure 3. Complete Retinal Blindness in LAB Mice

(A) Optomotor responses (OMR) measured in B6, CD1, HAB, NAB, LAB ($N=12$, each) under 500 lux. Inset shows a HAB animal within the OMR setup. Significance values obtained by 1-way ANOVA followed by Newman Keuls Multiple Comparison are indicated by asterisks compared to LAB or by hashes for B6 compared to all other mouse lines. (B) Simplified overview of the setup for measuring electroretinography in the anesthetized mouse. The b-wave is typically associated with the activity of Müller and ON bipolar cells. (C) Electroretinograms of B6, CD1, HAB, NAB, LAB ($N=6$, each) measured at scotopic and photopic conditions a three different flash intensities. Quantification of (D) scotopic ERG and (E) photopic measurements. Asterisks indicate significant group effect obtained by 2-way ANOVA followed by Bonferroni post hoc test. (F) Histological analysis of 30 μm retinal sections of B6, CD1, HAB, NAB, LAB ($N=3$, each, right eye only) stained with haematoxylin and eosin. IS/OS inner/outer photoreceptor segments; ONL outer nuclear layer; OPL outer plexiform layer; INL inner nuclear layer; IPL inner plexiform layer; GCL ganglion cell layer. (G) Polymerase chain reaction (PCR) screening for *Pde6b*^{rd1+/+} allele, *retinal degeneration 1*; bp base pair. Significance values are indicated by asterisks and hashes (details for the statistical tests are given in the respective part of the figure legend): * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. LAB; ### $p<0.001$ vs. CD1, HAB, LAB and NAB. Values are given as mean \pm SEM.

277 Reversing the Low-anxiety Phenotype of LAB Mice

278 The severe deficit in avoiding approaching threats of LAB mice during the Robocat task is explained
 279 by their retinal degeneration. However, using the IndyMaze, where retrievals (after the ball had hit
 280 the animal) were also counted as fear responses, it became obvious that LAB mice also showed a

281 decreased responsivity towards tactile stimuli. Therefore, it was necessary to determine whether
282 this behavioral abnormality can be ascribed to differential activity in a certain brain area. In order
283 to investigate the tonic neuronal activity changes in LAB mice compared to NAB, we employed
284 manganese-enhanced magnetic resonance imaging (MEMRI) in HAB ($N=31$), NAB ($N=26$) and LAB
285 ($N=30$) mice (FDR $p<0.001$, cluster extent >20), using a 3-level full-factorial design voxel-wise analysis
286 (for the complete MEMRI data set *see*). The results obtained by pairwise comparison of MEMRI
287 data (i.e., HAB vs. NAB, LAB vs. NAB) suggested, among others, a decreased accumulation of
288 manganese within the ventral parts of the deep and intermediate layers of the superior colliculus
289 (lateral to the periaqueductal gray) in LAB mice (Fig 4A). This structure receives dense inputs from
290 the primary and secondary somatomotor areas (Allen Brain Atlas, Connectivity, exps. #180719293,
291 #180709942). In order to assess the functional relationship of this brain region in the generation
292 of the LAB behavioral phenotype, the recently developed DREADD approach (*Armbruster et al.,*
293 **2007**) was employed. The activating DREADD hM3Dq fused to the reporter protein mCherry was
294 expressed under the control of the CaMKII α promoter using adeno-associated viral vectors (AAV5-
295 CaMKII α -hM3Dq-mCherry, $N=11$) or the control virus (AAV5-CaMKII α -mCherry, $N=12$) within the SC
296 (ML ± 0.9 mm, AP -3.64 mm, DV -1.75 mm). An exemplary image of the virus expression is shown in
297 Fig 4B. This approach resulted in the labeling of the entire SC (for detailed histological verification
298 *see* A). After an incubation period of >5 weeks, all animals were subjected to the IndyMaze. On the
299 testing day each animal was injected (i.p.) with 1 mg/kg CNO 45 minutes before the trial (as both,
300 experimental and control animals, received the same amount of CNO, the previously discovered
301 side-effects of converted clozapine (*Gomez et al., 2017*) cannot explain the behavioral changes).
302 Experimental animals expressing hM3Dq showed a significantly (Mann-Whitney $U_{n_1=94, n_2=182}=16.00$,
303 $p=0.0023$) increased latency to leave the start compartment (1282.0 ± 185.4 s vs. 265.5 ± 113.6
304 s; Fig 4C). Moreover only 64% of hM3Dq animals left the start compartment (Fig 4D) within 30
305 minutes (Fisher's exact, $p=0.0373$). Also the latency for end exploration was increased (216.0 ± 62.7 s
306 vs. 51.0 ± 25.3 s; Mann-Whitney U test; $U_{n_1=86, n_2=104}=8.00$, $p=0.0046$; Fig 4E). The fear responsivity
307 (Fig 4F) was increased to 71% of mice transfected with hM3Dq, compared to 9% in mCherry
308 controls (Fisher's exact, $p=0.0095$). Next, we tested whether this pharmacogenetically augmented
309 defensive response pattern, is also reflected by changed behavior on the EPM (one week after
310 IndyMaze task, CNO injection 45 min prior to experiment; Fig 4G). Similar to the emergence
311 component of the IndyMaze, hM3Dq animals treated with 1 mg/kg CNO showed an increased
312 (85.6 ± 12.9 s vs. 27.4 ± 2.4 s) latency to access the open arm ($U_{n_1=69.5, n_2=183.5}=3.500$, $p=0.0002$). This
313 was accompanied by a decreased percentage of time spent on the open arms (61.9 ± 4.2 % vs.
314 76.0 ± 3.3 %, $U_{n_1=183, n_2=93}=27.00$, $p=0.0178$), an increased percentage of time spent in the closed
315 arms (33.6 ± 4.3 % vs. 18.3 ± 3.0 %, $U_{n_1=100.5, n_2=175.5}=22.50$, $p=0.0081$) as well as decreased locomotor
316 activity (7.1 ± 0.7 m vs. 9.7 ± 1.0 m, $U_{n_1=179, n_2=97}=31.00$, $p=0.0337$). Time in center was unaffected
317 (*see* B). The partially reverted behavioral phenotype of LAB mice on the EPM could be explained
318 by an increased passivity due to nonspecific effects of the active DREADD. However, the increased
319 number of active risk assessment behavior (Fig 4H) in hM3Dq animals (13.0 ± 1.4 vs. 4.0 ± 0.7) points
320 towards higher levels of defensive responses ($U_{n_1=56.5, n_2=174.5}=1.5$, $p=0.0002$). In addition, also the
321 duration of SAPs was increased (8.9 ± 2.4 s vs. 2.0 ± 0.6 s) within the first 5 minutes of the EPM
322 (hM3Dq \times time: $F_{2,38}=3.59$, $p=0.0375$, 2-way rmANOVA). Together, these results show that elevation
323 of neuronal activity within the SC increased open-arm avoidance and risk assessment behavior
324 even in blind LAB animals. Moreover, the pharmacogenetic stimulation of the SC could restore in
325 part, the deficits in defensive responses to tactile stimuli.

326 **Reversing the High-anxiety Phenotype of HAB Mice**

327 Similar to LAB mice, we used the MEMRI approach to identify the neural circuitry which potentially
328 underlies the maladaptive defensive response pattern and increased open-arm avoidance behavior
329 in HAB mice. A prominent brain structure found to exhibit increased manganese accumulation,
330 was the ventrolateral, lateral (l) and dorsolateral (dl) periaqueductal gray (Fig 5A; for the complete

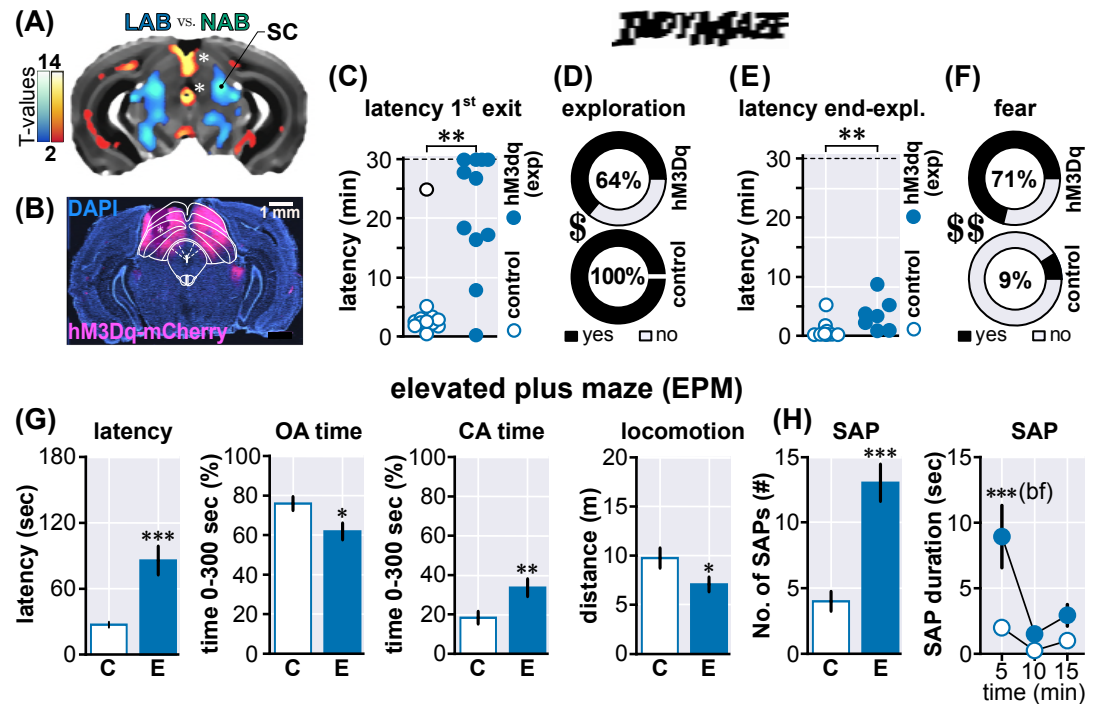


Figure 4. Reversing the Low-anxiety Phenotype of LAB Mice

(A) Manganese enhanced MRI (MEMRI) of LAB ($N=30$) vs. NAB ($N=26$) animals exhibited a significantly decreased accumulation of Mn^{2+} within the deep and intermediate layers of the superior colliculus of LAB. Warm colors indicate increased accumulation, cold colors indicate decreased accumulation in LAB as compared with NAB. The color brightness indicates the significance values. Asterisks mark signal artifacts within the aqueduct and above the superior colliculus due to line differences in brain templates. **(B)** Exemplary brain section at approximately the same slice location as the MEMRI data, depicting extent of viral expression (magenta) at the level of the superior colliculus. Shown in cyan is the nuclear 4',6-diamidino-2-phenylindole (DAPI) counterstain. Overlaid are the outlines of the SC and PAG. Asterisk marks tissue lesion which occurred during sectioning. The effect of hM3Dq activation within the SC was studied using the IndyMaze task. Shown is the latency to first exit **(C)**, the percentage of animals which explored the arena at all **(D)**, latency to end-exploration **(E)** and the percentage of animals which showed a fear response to the ball **(F)**. All animals were treated with 1 mg/kg clozapine-*N*-oxide (CNO) 45 minutes before the test. **(G)** In addition the same animals were tested for the behavior on the EPM (30 minutes), and latency to emerge, open arm (OA) time, closed-arm (CA) time and locomotion was assessed within the first 5 minutes. **(H)** Moreover the active risk assessment parameters, i.e. the total number of stretched-attend postures (SAP) and the duration of SAPs over time (0-15 min) were scored. Asterisks indicate significance values obtained by Mann-Whitney test if not stated otherwise, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; dollar signs indicate significance values obtained by Fisher's exact tests, \$ $p < 0.05$, \$\$ $p < 0.01$. Significance values obtained by 2-way rmANOVA, followed by Bonferroni post-hoc test are indicated with *bf*. Values are given as mean \pm SEM.

MEMRI data set see). In order to assess the functional relationship of the PAG in the generation of the HAB behavioral phenotype, we implanted guide cannulae targeting the dl/IPAG (ML ± 0.6 mm, AP -4.25 mm, DV -2.45 mm, needle protruded 500 μ m) and injected 53.24 ng/100 nl (per hemisphere) of fluorescently labeled muscimol (MUSC), a potent GABA_A-agonist (45 minutes before each experiment) which is comparable to 10 ng in 100 nl of ordinary muscimol. An exemplary image depicting the muscimol diffusion is shown in Fig 5B. The extent of muscimol diffusion of all animals ($N=11$) is shown in A) and comprised besides the IPAG, the dlPAG and partly the deep and intermediate layers of the SC. In order to test whether increased GABAergic signaling within the IPAG changes the extreme open-arm avoidance behavior of HAB mice, we have tested vehicle (aCSF, $N=6$) or MUSC ($N=5$) treated HAB mice (one VHC and two MUSC animals have been excluded from analysis due to deficient infusion) for their behavior on the EPM (Fig 5C-H). While only 20% of VHC treated animals accessed the open arm, all MUSC animals readily did so (Fisher's exact, $p=0.0152$; Fig 5C+D). Further, MUSC treated animals spent significantly ($U_{n1=16, n2=50}=1.000$, $p=0.0116$) more time on the open arm (44.4 ± 9.4 % vs. 3.2 ± 3.2 %; Fig 5E), less time in the closed arm (45.0 ± 9.3 % vs. 88.1 ± 5.8 %, $U_{n1=44, n2=22}=1.000$, $p=0.0087$; Fig 5F) and showed increased locomotion (17.6 ± 2.2 m vs. 8.0 ± 1.2 m, $U_{n1=15, n2=61}=0.0$, $p=0.0043$; Fig 5G). Time in center was unaffected (see B). These observations indicate a decrease in open-arm avoidance, which however, could be confounded by the increased activity. Therefore a decrease of active risk assessment (shown earlier to be sensitive to systemic diazepam treatment; see Fig 1F) in MUSC treated HAB mice (Fig 5H), namely number of

350 SAPs (MUSC 5.0 ± 3.5 vs. VHC 19.6 ± 3.4 , $U_{n1=45, n2=21} = 0.0$, $p = 0.008$) supports the decrease in defensive
 351 responses. Moreover, the duration of SAPs was significantly decreased within the first 5 minutes of
 352 the EPM task with significant group effect ($F_{1,9} = 13.71$, $p = 0.0049$, 2-way rmANOVA; Fig 5H). Finally, all
 353 animals were tested two times on two consecutive days for their disposition to vocalize during a
 354 5 min TST, using a crossover design. Half of the animals received either VHC or MUSC treatment,
 355 which was swapped at the following day. Whereas 86% of VHC treated HAB mice emitted at least
 356 one sonic call during a 5 min TST, none of the MUSC treated animals vocalized (Fisher's exact,
 357 $p < 0.0001$; Fig 5I). All calls were in the sonic range. Fig 5J (*upper panel*) shows the spectral analysis
 358 of sonic vocalizing HAB mice (two mice have been excluded due to their low disposition of only
 359 short calls). Evidently HAB mice vocalize at a dominant frequency of 4090 Hz with a strong 1st
 360 harmonic at 8180 Hz. All recordings were carried out using a USV transducer and were scored
 361 online using the heterodyne headphone output, thereby we can exclude that MUSC treated animals
 362 vocalized in the ultrasonic range only. Fig 5J (*lower panel*) shows an exemplary sonic call with the
 363 dominant frequency in the 4 kHz range, and formant harmonics up to approx. 16 kHz. In some
 364 calls (white asterisks) we can see that these harmonics even range up to 40-50 kHz, however these
 365 signals do not resemble any typical rodent ultrasonic call. These results, indicate an increased
 366 tonic activation of the PAG in HAB mice, which precipitates as an exaggerated open-arm avoidance
 367 behavior accompanied by a strong disposition to emit sonic calls, which could be reverted by low
 368 doses of muscimol.

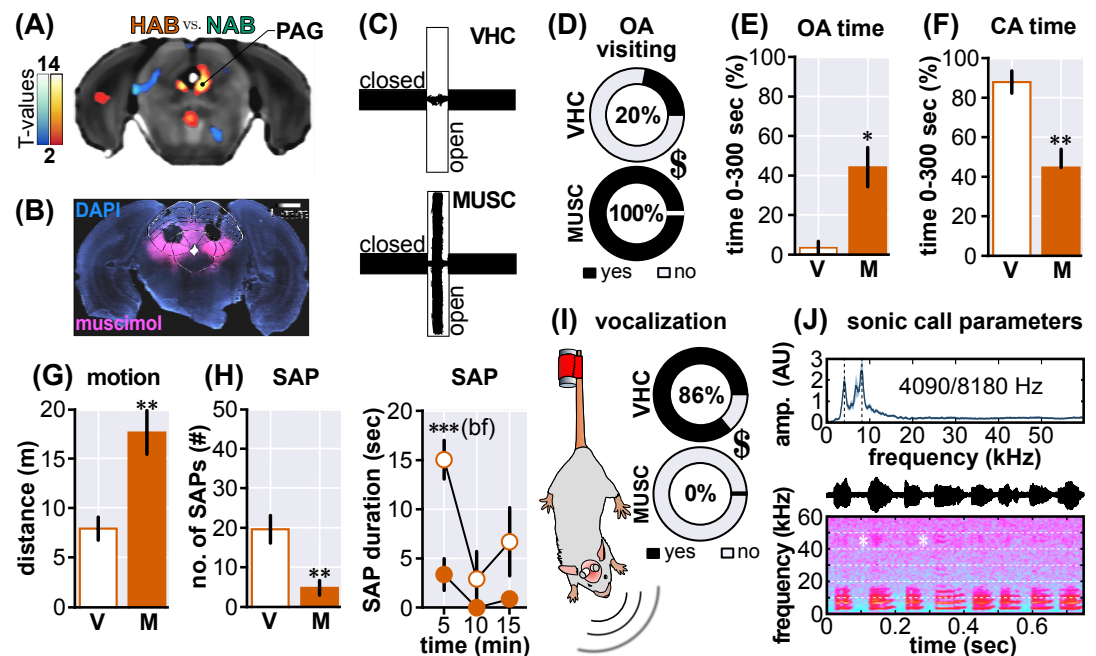


Figure 5. Reversing High-anxiety Phenotype of HAB Mice

(A) Manganese enhanced MRI (MEMRI) of HAB ($N=31$) vs. NAB ($N=26$) animals showed a significantly increased accumulation of Mn^{2+} within the periaqueductal gray of HAB. (B) Exemplary brain section at approximately the same slice location as the MEMRI image, depicting extent of fluorescently labeled muscimol (MUSC) diffusion (magenta) at the level of the periaqueductal gray. The nuclear DAPI counterstain is shown in cyan and overlaid by the outlines of the SC and PAG. Asterisk marks tissue lesion due to cannula placement. (C) Exemplary movement trace of a vehicle (VHC) and MUSC treated HAB mouse on the EPM. VHC ($N=5$) or MUSC ($N=6$) treated HAB animals were tested for their behavior on the EPM (30 minutes), and the percentage of open arm (OA) visiting animals (D), OA time (E), closed-arm (CA) time (F) and locomotion (G) was assessed within the first 5 minutes. (H) Moreover the active risk assessment parameters, i.e. the total number of stretched-attend postures (SAP) and the duration of SAPs over time (0-15 min) were scored. (I) Finally, animals ($N=14$) have been treated with VHC and MUSC in a crossover design and subjected to a 5 min tail-suspension test (see cartoon), in order to assay the disposition to vocalize. (J) *Upper panel*: Spectral analysis of vocal call emitted by HAB ($N=10$). Depicted is the average (black) together with the SEM (blue). Dashed horizontal lines indicate the dominant frequency at 4090 Hz and the first harmonic at 8180 Hz. *Mid panel*: Exemplary call of a HAB animal; hull curve of raw signal. *Lower panel*: Sonogram of the same call. Note the formant structure of the harmonics. Asterisks denote rare and slight ultrasound artifacts within the 40-50 kHz range, which occur due to the expelled air itself. Asterisks indicate significance values obtained by Mann-Whitney test if not stated otherwise, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; dollar signs indicate significance values obtained by Fisher's exact tests, \$ $p < 0.05$, \$\$ $p < 0.01$. Significance values obtained by 2-way rmANOVA, followed by Bonferroni post-hoc test are indicated with *bf*. Values are given as mean \pm SEM.

369 Discussion

370 The inner feelings during states of anxiety, fear and panic of laboratory animals are not accessible
371 to the experimenter. Instead one has to rely on behavioral and physiological readouts. Due to
372 the rather continuous nature of the behavioral expression of anxiety, fear and panic, these states
373 appear as a function of the animals defensive survival circuits. While this does not preclude a
374 classification of the observed measures, it eliminates the possibility to ascribe a certain inner state
375 to a behavioral category. In the description of the results presented in this study, we have limited
376 ourselves to the use of *avoidance* for situations where the animal controls its exposure to a threat
377 (emergence tasks), *active risk assessment* for the display of stretched-attend postures and *defensive*
378 *responses* for directed and undirected flight and tonic immobility (freezing).

380 Previous studies investigating the neuropharmacological basis of altered open-arm avoidance
381 of inbred mouse strains using the EPM, report a consistent dose-dependent *anxiolytic* effect of
382 systemically administered benzodiazepines (Rodgers et al., 1992; Cole and Rodgers, 1995; Holmes
383 and Rodgers, 1999; Griebel et al., 2000) which emphasizes the predictive validity of this testing
384 situation. More recent studies which assess the involvement of specific brain areas in the expression
385 and regulation of open-arm avoidance in rats and mice implicate the prefrontal cortex (PFC)
386 (Adhikari et al., 2010, 2011; Kumar et al., 2013), bed nucleus of the stria terminalis (BNST) (Kim et al.,
387 2013), lateral septum (LS) to anterior hypothalamic area (AHA) projection (Anthony et al., 2014),
388 medial septum (MS) (Shin et al., 2009; Zhang et al., 2017), septo-habenular pathway (Yamaguchi
389 et al., 2013), basolateral amygdala (BLA) (Sorregotti et al., 2018) and specifically its projections
390 towards the central nucleus of the amygdala (CeA) (Tye et al., 2011) and ventral hippocampus (vHPC)
391 (Felix-Ortiz et al., 2013) and PFC (Felix-Ortiz et al., 2016), vHPC to PFC projection (Padilla-Coreano
392 et al., 2016), vHPC-lateral hypothalamic area (LHA) projection (Jimenez et al., 2018), habenula (Hb)
393 (Pang et al., 2016), interpeduncular nucleus (IPN) (Zhao-Shea et al., 2015), laterodorsal tegmentum
394 (LdT) (Yang et al., 2016) but also the PAG (Santos et al., 2003; Netto and Guimarães, 2004; Borelli
395 and Brandão, 2008; Lima et al., 2008; Campos and Guimarães, 2009; Mendes-Gomes and Nunes-
396 de Souza, 2009; Terzian et al., 2009; Muthuraju et al., 2016) and the SC (Muthuraju et al., 2016). It
397 is clear that all these brain structures cannot mediate the same types and aspects of avoidance
398 behavior (e.g. social, olfactory, visual & auditory cues), but nevertheless they all modulate the
399 same behavioral readout. While this broad spectrum of potentially involved circuits might be
400 advantageous for initial behavioral screening purposes, the interpretation of the observed behavior
401 on the EPM demands extra care. Consequently, referring to this behavior as *anxiety-like* is an
402 oversimplification. In this study we presented data obtained from mouse lines which were generated
403 under the simple assumption that the level of open-arm avoidance is a proxy for *anxiety* (Krömer
404 et al., 2005).

405 It has to be noted that similar to the bidirectional selective breeding for open-arm avoidance
406 in mice, there has been an earlier approach in rats which resulted in high-anxiety and low-anxiety
407 behaving animals (for review see Landgraf and Wigger, 2003; Landgraf et al., 2007). However, even
408 though the findings obtained with HAB/LAB rats are highly relevant in the face of preclinical anxiety
409 research, their discussion is beyond the scope of this study.

410
411 We have shown that the open-arm avoidance phenotypes of two mouse-lines which have
412 been selectively bred for extremes in so-called *anxiety-like* behavior (HAB and LAB mice), based
413 on their behavior on the EPM, are accompanied by paralleled changes in the level of defensive
414 responses. This was demonstrated using two novel multi-sensory behavioral paradigms (Robocat,
415 IndyMaze) which allowed the repeated assessment of innate escape behavior towards an approach-
416 ing threatening stimulus. Further, we have discovered that LAB mice lack a functional retina due
417 to a homozygous mutation in the *Pde6b*^{rd1+/+} allele which is indicative of the *retinal degeneration*
418 *1 (rd1)* mutation and leads to blindness shortly after birth. Nonetheless, applying MEMRI-guided

419 *in-vivo* neuronal circuit inquiry using the activating DREADD hM3Dq in the SC of LAB mice, we
420 were able to demonstrate that increasing the neuronal activity within a midbrain multi-sensory
421 integration circuit is sufficient to increase the level of innate defensive responses even in blind
422 animals which ultimately precipitates as increased open-arm avoidance on the EPM. Further, using
423 a similar approach but employing local injections of the potent GABA_A-agonist muscimol into the
424 PAG of HAB mice, we could show that also in this case a modulation of the neuronal activity within
425 the midbrain survival circuits is sufficient to reverse the dominant open-arm avoidance phenotype.

426 **The Multidimensional Nature of Selective Breeding**

427 Both bottom-up (which start from a defined genetic alteration or neuronal subpopulation or brain
428 structure) and top-down approaches (which start from a distinct behavioral phenotype) hold the
429 promise to decipher the molecular and cellular basis of anxiety-like behavior (*Anderzhanova et al.,*
430 *2017*). The latter approach includes selective breeding and allows to study behavioral phenotypes
431 on a polygenic background, which resembles the situation in most psychiatric diseases (*Landgraf*
432 *et al., 2007; Anderzhanova et al., 2017*). It assumes that the resulting extremes in anxiety-like
433 behavior reflect extremes in the normal distribution of the same behavioral trait (*Sartori et al.,*
434 *2011b*). Accordingly, a direct comparison between the two extremes is expected to provide an
435 optimal signal-to-noise ratio to disentangle molecular and cellular correlates of the phenotype.
436 In fact, activity propagation through the amygdala circuit seems to support a dimensional shift
437 from HAB via NAB to LAB phenotype (*Avrastos et al., 2013*), and many behavioral readouts show a
438 similar pattern (see Table 1). The present study, however, demonstrates that this strategy might
439 be misleading if not entirely wrong: First, measurements of differences in activity-dependent
440 accumulation of Mn²⁺ did not reveal a single brain structure with bidirectional changes in signal
441 intensity in HAB and LAB compared to NAB mice. Second, we identified impairments in sensory
442 perception as a putative source of threat neglect in LAB mice. The *rd1* mutation freely segregates
443 in many mouse strain populations, including CD1 (the ancestor strain used for the initial step of
444 selective breeding). It stands for a nonsense mutation in the photoreceptor phosphodiesterase
445 6b (Pde6b). In case of homozygosity, the recessive mutation results in photoreceptor loss and
446 retina degeneration. It is conceivable that the selective breeding of LAB is based, at least in part, on
447 co-selection for *rd1* and the resulting physical blindness. Due to the lack of material, we cannot
448 trace back the time point of first occurrence of homozygosity since the establishment of the LAB
449 line more than 15 years ago (*Krömer et al., 2005*). In any case, data obtained in the past by direct
450 comparison of LAB vs. HAB have to be (re-)interpreted with great care.

451 **In-vivo Imaging**

452 We employed *in vivo* MEMRI imaging to investigate the neural basis of extremes in anxiety-like
453 behavior. Other than expression of immediate early genes or accumulation of radioactive derivatives
454 of glucose which measure phasic changes in neuronal activity upon acute exposure to a threatening
455 situation, repeated injections of MnCl₂ are expected to result in intracerebral accumulation of
456 Mn²⁺ also in cells with tonic (i.e., lasting) changes in neuronal activity. Importantly, MEMRI has the
457 potential to non-invasively map whole-brain activity (*Bangasser DA, 2013; Bissig and Berkowitz,*
458 *2009; Chen et al., 2013, 2007; Eschenko et al., 2010; Hoch et al., 2013; Laine et al., 2017; Tang*
459 *et al., 2016*). Mn²⁺ enters active neurons through voltage-gated calcium channels (*Drapeau and*
460 *Nachshen, 1984*) (e.g. Ca_v1.2; (*Bedenk et al., 2018*)), is transiently kept intracellularly (*Gavin et al.,*
461 *1990*) and preferentially accumulates in projection terminals (*Bedenk et al., 2018*), which suggests
462 its application for connectome analyses. Although our animals were not explicitly challenged, it has
463 to be noted that the injection procedure per se may act as an acute stressor which triggers distinct
464 neural responses in the different mouse lines. In fact, mice showed a prominent corticosterone
465 secretion following treatment with Mn²⁺, which declined over the course of repeated injections
466 (*Grünecker et al., 2010*).

467 Voxel-wise comparisons revealed a variety of brain structures with lower or higher Mn²⁺ accu-

468 mulation in HAB or LAB vs. NAB. A detailed discussion of each of them is far beyond the scope
469 of the present study. We wish to mention only a few prominent (and unexpected) findings such
470 as the globus pallidus (GP) in LAB and the septo-hippocampal system in HAB. The GP is primarily
471 associated with motor and associative functions (*Deniau et al., 2010*). However, it seems to play an
472 important role also in the expression of aversive behaviour, including fear and anxiety (*Talalaenko*
473 *et al., 2008*). Local administration of serotonin and glutamic acid into the GP effectively suppressed
474 anxiety-like behaviour in the threatening situation avoidance test (*Talalaenko et al., 2008*), and
475 downregulation of the corticotropin releasing factor receptor 1 led to an anxiogenic effect (*Sztain-*
476 *berg et al., 2011*). Further clinical evidence for crucial involvement of the GP in anxiety mediated
477 behaviour is given by the fact that deep brain stimulation within the GP was accompanied by a
478 decrease of anxiety symptoms in depressive patients (*Kosel et al., 2007*) as well as patients suffering
479 from Parkinson's disease (*Tröster et al., 1997*). We cannot entirely rule out that the differences in GP
480 activity may relate to line differences in general locomotor activity (*Yen et al., 2013, 2015*). However,
481 one would assume that increased motor activity, if at all, would lead to increased accumulation of
482 Mn^{2+} in the motor network, resulting in an effect of order LAB > NAB not LAB < NAB.

483 Our finding of increased activation of the septal-hippocampal system in HAB is particularly
484 interesting, given its suggested involvement in generalized anxiety disorder (*Gray and McNaughton,*
485 *2000*). Septal lesions were reported to increase the time spent on the open arm of the EPM and to
486 decrease the time spent burying the shock probe (*Menard and Treit, 1996*), and the local adminis-
487 tration of the arginine vasopressin receptor antagonist into the septum of rats led to an increased
488 time spent on the open arm of the EPM (*Liebsch et al., 1996*). Septal neurons which express the
489 corticotropin releasing factor receptor 2 project to the hypothalamus and promote anxiety-like
490 behavior (*Anthony et al., 2014*). Also the hippocampus has been implicated in anxiety-like behavior
491 (*Bannerman et al., 2004*), in particular its ventral part (*Felix-Ortiz et al., 2013; Padilla-Coreano et al.,*
492 *2016; Jimenez et al., 2018*). Therefore, it is tempting to assume that the higher activity status of the
493 hippocampus in HAB mice indicated by increased Mn^{2+} accumulation causally links to exaggerated
494 fear and anxiety-like behavior shown by the animals. This interpretation is supported by PET-studies
495 in rhesus monkey which report increased brain activity in the hippocampus of hyperanxious animals
496 (*Oler et al., 2010*). Interestingly, also LAB mice show higher Mn^{2+} accumulation in the hippocampus
497 formation, which seems to contradict our interpretation. However, we observed a prominent signal
498 at the level of the dorsal dentate gyrus, which has been associated with hyperlocomotion and
499 decreased anxiety before (*Kheirbek et al., 2013*), thus resembling the LAB phenotype (*Yen et al.,*
500 *2013*).

501 **Midbrain Structures Control the Level of Open-arm Avoidance, Risk Assessment** 502 **and Defensive Behavior**

503 Among the many brain structures with different accumulation of Mn^{2+} , there were also parts of
504 the midbrain/tectum, which showed reduced (e.g., superior and inferior colliculus in LAB mice) or
505 enhanced (PAG in HAB mice) signal intensities. The superior colliculus is a multimodal sensory-
506 motor structure that receives inputs from the retina and somatosensory cortex (*King, 2004; Shi*
507 *et al., 2017*). Efferences from the superior colliculus trigger a variety of defensive responses (*Shang*
508 *et al., 2015; Evans et al., 2018*). Therefore, the reduced activity status of the superior colliculus may
509 reflect both reduced sensory inputs (i.e., threat detection; (*Almada et al., 2018*)) and reduced threat
510 responding. Physical blindness alone is insufficient to explain the behavioral phenotype in the
511 IndyMaze (where LAB mice failed to show flight responses even after contact with the styrofoam ball)
512 and on the EPM (low level of risk assessment). Indeed, we could reduce the 'emotional blindness' by
513 chemogenetic activation of the superior colliculus. In HAB, the enhanced Mn^{2+} accumulation spans
514 the entire caudal part of the PAG and resembles the enhanced expression of c-Fos in HAB mice
515 which had been confined to an open arm of the EPM (*Muigg et al., 2009*). The increased activity in
516 ventral parts of the PAG correspond to the prevalence of HAB mice for showing passive defensive
517 responses (*Bandler R, 2000; Tovote et al., 2015*)(see also Table 1). The increased activity in dorsal

518 parts is more surprising, given their association with active defensive responses (**Bandler R, 2000**).
519 Only recently we could demonstrate that HAB mice show exaggerated active fear to an approaching
520 robo-beetle (**Heinz et al., 2017**), which is in accordance with the exaggerated flight responses to
521 the Robocat shown in the present study. Remarkably, inactivation of the dorsal PAG led to the
522 most striking changes in EPM behavior observed so far in this mouse line. This also applied to the
523 reduction in risk assessment and the complete absence of defensive vocalization.

524 **Of Fear and Anxiety**

525 In animals, anxious states are prototypically assessed in exploration- or interaction-based tasks
526 which involve approach-avoidance conflicts (**Belzung and Griebel, 2001; Millan, 2003; Cryan and**
527 **Holmes, 2005; Sousa et al., 2006**). Avoidance measures alone are insufficient to describe the behav-
528 ioral phenotype, since they might be confounded by alterations in exploratory drive. Therefore, it is
529 strongly recommended to additionally assess ethobehavioral parameters which reflect approach
530 behavior towards a (potentially) threatening environment/object (**Gray and McNaughton, 2000**).
531 Here we report bidirectional changes in risk assessment (**McNaughton and Corr, 2018**) on the EPM
532 in HAB and LAB vs. NAB mice, which were sensitive to diazepam treatment and could be reverted
533 by pharmacological or chemogenetic interventions at midbrain/tektal structures. Open-arm explo-
534 ration, in contrast, was insensitive to diazepam and, thus, seems to be less suited as a measure of
535 anxiety-like behavior. This might be ascribed to the stringent selection process over the generations
536 (**Krömer et al., 2005**), and the threat intensity due to the combination of height and open spaces.
537 Accordingly, during the first phase of the IndyMaze, when mice have to leave the home compart-
538 ment to explore the hollow way engulfed by side and end arms, HAB mice showed a strong increase
539 in emergence latencies. This time, however, this parameter was sensitive to diazepam treatment,
540 possibly because of a less threatening impact of the test situation. Importantly, HAB mice readily
541 explored the entire setup, once they had left the home compartment. Therefore, differences in
542 EPM or IndyMaze exploration cannot be ascribed to a general lack in motivation/exploratory drive
543 or locomotor behavior, but to state anxiety. To study the consequences of extremes in anxiety-like
544 behavior on defensive responses to explicit threat, we decided to develop ethobehavioral tasks
545 (**Pellman and Kim, 2016**), which allow for the measurements of active defensive responses as a
546 function of defensive distance and to judge their adaptive vs. maladaptive nature. In the IndyMaze,
547 mice were confronted with an approaching styrofoam ball, which spans the entire width of the
548 hollow way. Whereas both HAB and NAB escaped from the ball, the behavior of LAB mice was
549 clearly maladaptive, since virtually all mice were overrun by the ball at least once (without physical
550 harm). As discussed before, increased neuronal activity in the superior colliculus reestablished
551 flight behavior in the majority of the animals, demonstrating that not exclusively sensory deficits (i.e.,
552 physical blindness) can explain the deficits in defensive behavior. A similar picture emerged from
553 the Robocat exposure, which resembles the robogator described before (**Choi and Kim, 2010; Amir**
554 **et al., 2015; Pare and Quirk, 2017**). Again, LAB mice were at high risk to collide with the Robocat.
555 This time, also the behavior of HAB mice turned out to be maladaptive, since no mouse could
556 bypass the Robocat even if not at risk to collide with it. Together with our previous observation of
557 increased avoidance of an approaching robo-beetle (**Heinz et al., 2017**), this finding suggests that
558 selection for high levels of anxiety-like behavior on the EPM coincides with exaggerated defensive
559 responses, both passive (see Table 1) and active.

560 **Conclusion**

561 Using well-established mouse lines with extremes in anxiety-like behavior, we demonstrate that
562 extremes in anxiety coincide with (i) extremes in defensive responses to an approaching threat and
563 (ii) tonic changes in neuronal activity, among others in midbrain/tektal structures, (iii) which – if
564 reverted – ameliorated both fear- and anxiety-like behavior. In addition, we provide evidence for (iv)
565 the multidimensional nature of increased vs. decreased defensive behavior, which may include
566 deficits in sensory perception. Our results challenge the uncritical use of the anthropomorphic terms

567 anxiety or anxiety-like for the description of mouse behavior on the EPM or in other exploration-
568 based tasks (*for review see Ennaceur, 2014*), as they imply higher cognitive processes, which are
569 not necessarily in place. The explicit fear of height (acrophobia) and/or open spaces (agoraphobia)
570 sufficiently explains the lack of open-arm exploration. The recently initiated discussion about the
571 uncritical use of the term 'fear' where 'threat' would be more appropriate (*LeDoux, 2012*) has forced
572 the scientific community to reconsider its terminology, even though the term 'fear' still keeps its
573 merits (*LeDoux, 2014, 2017*). We face a similar if not more eminent problem, if we uncritically
574 use the term 'anxiety' in translational studies on animal behavior. Instead, we should describe
575 defensive responses as they are, preferentially along the continuum of the *predatory imminence*
576 *model* (*Perusini and Fanselow, 2015*).

577 **Methods and Materials**

578 **Animals**

579 Adult (3-8 months), male mice of the following strains have been used: C57BL/6N (B6) ($N=12$), HAB
580 ($N=154$), NAB ($N=76$), LAB ($N=99$), CD1 ($N=12$), resulting in a total number of 353 animals. All animals
581 were bred in the animal facilities of the Max Planck Institute of Biochemistry, Martinsried, Ger-
582 many. The animals were group-housed (2-4 animals per cage) under standard housing conditions:
583 12h/12h inverted light-dark cycle (light off at 8AM), temperature $23\pm 1^\circ\text{C}$, food and water *ad libitum*.
584 Experimental procedures were approved (55.2-1-54-2531: 44-09, 188-12, 142-12, 133-06, 08-16)
585 by the State of Bavaria (Regierung von Oberbayern, Munich, Germany). Animal husbandry and
586 experiments were performed in strict compliance with the European Economic Community (EEC)
587 recommendations for the care and use of laboratory animals (2010/63/EU). All efforts were done to
588 minimize the number of experimental subjects and to preclude any animal suffering.

589 **Drugs**

590 The anxiolytic diazepam (Diazepam-Lipuro[®], BRAUN Melsungen, Germany) was diluted in physiolog-
591 ical saline (vehicle) and injected systemically (1 mg/kg, i.p.) using a volume of 100 μl per 10 g body
592 weight. Clozapine-*N*-oxide (CNO, Tocris #4936) was dissolved in dimethyl sulfoxide (DMSO, Sigma-
593 Aldrich, #472301) at a stock concentration of 75 mM and stored at -20°C . The final concentration of
594 CNO was 292 μM (1 mg/kg at 100 μl per 10 g body weight) in saline ($<0.5\%$ DMSO). Muscimol MUSC
595 (Sigma-Aldrich, #M1523) and fluorescently-labeled muscimol (fMUSC) (BODIPY[®]TMR-X conj. Thermo
596 Fisher Sc. M23400) was dissolved in artificial cerebrospinal fluid (aCSF) (*Baarendse et al., 2008*).
597 MUSC itself has a molecular weight of 114.1 g/mol whereas the fMUSC (MW 607.46 g/mol) is 5.324x
598 heavier. In previous experiments with MUSC, we found a concentration of 10 ng/100 nl (876.4 μM)
599 most effective, therefore we have used 53.24 ng/100 nl fMUSC to achieve the same physiological
600 effect. As the fMUSC is poorly water-soluble, we dissolved 1 mg in 1.878 ml aCSF to reach a final
601 ready to use concentration of 876.6 μM . Whereas the EPM experiments were conducted using
602 MUSC, the vocalization experiments only involved fMUSC. The vocalization experiment was carried
603 out using a crossover design: half of the animals received fMUSC on the first day, whereas the other
604 half received VHC (aCSF). On the next day the treatment was switched. 1-3h after the experiment
605 the animals which received fMUSC were transcardially perfused (4% PFA in PBS), whereas the
606 remaining animals received another injection of fMUSC on the following day and were also perfused
607 1-3h after the injection.

608 **Behavioral Tests**

609 **Elevated Plus Maze**

610 The elevated plus maze (EPM) apparatus consisted of two open (L30xW5 cm) and two closed
611 (L30xW5xH15 cm) arms which were connected via a central platform (L5xW5 cm). All parts of the
612 EPM were made of dark gray PVC. The apparatus was elevated 37 cm above a table (H50 cm), which
613 was placed in the center of the dim illuminated experimental room (5x4 m). The light intensity

614 (luminous flux) at the open arms was 7 lux. Before the experiment every subject was tested for the
615 disposition to emit sonic vocalizations by lifting them 3 times from the grid cage top (*Whitney, 1970*).
616 At the beginning of each trial, the animal was placed near the central platform facing a closed arm.
617 Each trial lasted for 30 minutes and was videotaped. The animals behavior was analyzed using a
618 behavioral tracking software (ANY-maze, Stoelting CO., USA) and the percentage of time spent on
619 the open (OA) and closed (CA) arm and the central zone (time in center) as well as the total distance
620 traveled were determined. In order to render these results comparable to other EPM experiments
621 found in the literature, the data (except for latency and stretched-attend postures, SAPs) of the
622 first 5 minutes of each trials is reported. Other behavioral parameters which were analyzed by
623 an experienced observer, blind to the experimental conditions, included number and duration of
624 SAPs within the first 15 minutes of each trial, and the latency for the first full entry to the open
625 arm (all four paws) within the entire 30 minutes exposure. After the trial the fecal boli on the EPM
626 apparatus were counted as a measure of autonomic arousal. In between the trials the apparatus
627 was cleaned with tap water containing detergent, and was subsequently dried with tissues.

628 Robocat Task

629 The Robocat (for a detailed explanation of the task *see*) has been inspired by the Robogator
630 (*Choi and Kim, 2010*). It is a four-wheeled robot (Lego Mindstorms), equipped with ultrasound
631 range finders and programmed to advance for 25 cm (speed 25 cm/s) once a movement has been
632 detected within the sensor range of 50 cm. Despite the name suggests, no extra effort has been
633 invested to disguise the robot as a cat, except two little cardboard ears. The task is conducted
634 within a longitudinal arena (H35×W50×L150 cm, whereby the robot is placed 125 cm away from
635 the start compartment (H35×W50×L12.5). The access to the arena is provided via a sliding door,
636 operated by the experimenter, and the natural exploratory drive (neither bait, nor food or water
637 deprivation used) ultimately leads to the mouse-robot encounter. Once the mouse triggers the
638 robot, its movements typically evoke a robust flight response and the mouse retrieves to the start
639 compartment. All animals were first pre-exposed to the entire setup with unrestricted access to
640 the arena (sliding door opened) in absence of the Robocat. On the following consecutive 3 days
641 each animal was subjected to habituation trials which consisted of 10 minutes acclimatization
642 within the start compartment (to enable the mice to form a home base), followed by 10 minutes of
643 free exploration in the arena, again without the Robocat. The test trial on day 4 was conducted in
644 identical manner, except that the Robocat was placed in the arena. During the test trial, the animals
645 typically activated the Robocat several times. All trials were videotaped and the behavior was
646 analyzed offline by an experienced observer, blind to the experimental conditions. The behavioral
647 readouts were flight (activation + retrieval), bypass (activation but tolerance to the approaching
648 Robocat which is bypassed by the animals) or collision, and were counted if observed at least once.
649 Only animals which activated the Robocat at least once were considered for analysis.

650 IndyMaze Task

651 Inspired by the movie *Indiana Jones and the Raiders of the Lost Ark* (*Spielberg and Marshall, 1981*), the
652 IndyMaze is conducted within a narrow, stretched arena (H35×W16×L150 cm), which was divided
653 into six equidistant (25 cm) sectors. To one end of the arena, a small custom-made plexiglass
654 cage (H30×W16×L25 cm), equipped with bedding material, was connected which served as a home
655 compartment. The arena itself was slightly tilted towards the home compartment and indirectly
656 lit (<10 lux). To enter the arena, the animals had to climb over a small barrier (height: 2 cm). This
657 prevented the animals from 'accidentally' dropping into the arena and forced them to explicitly
658 decide when to initiate its exploration. For the task, each animal was first placed into the home
659 compartment and was allowed for a maximal duration of 30 minutes to step (with four paws)
660 into the arena (latency 1st entrance). Once the animal had entered the arena, the time to reach
661 the last sector was noted (latency for end-exploration). After the end exploration, the animals
662 typically retrieved to the home compartment or were gently forced to do so by the experimenter.

663 With low latency the animals re-entered the arena but this time a styrofoam ball (\varnothing 15cm , 100 g)
664 was introduced at the last sector, which was allowed to roll (25 cm/s) towards the animal once it
665 passed the midline (75 cm). The animals either responded with (a) preemptive flight or a retrieved
666 once the ball hit them (both counted as defensive responses), or (b) they were overrun by the ball
667 (without any physical harm) and continued to explore the arena. The threat exposure part of the
668 behavioral paradigm was carried out for a maximal duration of 30 minutes or once the animals had
669 encountered the ball three times. The behavior was scored online by the experimenter during the
670 task unaware of the mouse line.

671 Optomotor Response

672 In order to assess the visual performance of male C57BL/6N, CD1, HAB, NAB and LAB mice ($N=12$),
673 the animals' optomotor response (*Abdeljalil et al., 2005*) has been tested using the rotating drum
674 task. The task is based on the mice's predisposition to fixate on moving vertical black/white stripes
675 and follow their rotation with short movement bouts, involving the entire head. By decreasing the
676 stripe width, higher visual acuity is necessary to resolve the stripes. The apparatus consisted of a
677 rotating cylinder (drum, \varnothing 33 cm, height 35 cm), whose inner walls were lined with an alternating
678 black/white stripe pattern using a stripe width of 2.88 cm, giving a spatial frequency of 0.05 cycles
679 per degree (cyc/deg, $r=16.5$ cm, arc length per black/white cycle 5.76, angle 20°). During the task, the
680 animals were placed within the center of the drum on a \varnothing 11.5 cm fan grid which was mounted 16
681 cm above the bottom. The rotation of the drum was controlled via a custom-built microprocessor-
682 based motor driving circuit which operated a geared motor. The rotational speed of the drum
683 was set to 2.5 rounds per minute (rpm). For the task the animals were placed into the drum for
684 1 minute to acclimatize (bright illumination 500 lux) and subsequently the drum started to rotate
685 for 60 seconds clockwise, followed by a 30 seconds break and then rotated in counter-clockwise
686 direction for additional 60 seconds. All experiments were videotaped and analyzed offline (blind to
687 the strains with same fur color, i.e. CD1, HAB, NAB and LAB), whereas every head movement was
688 scored as an optomotor response if it was directed into the same rotational direction as the drum.
689 This modified version of the original task (*Abdeljalil et al., 2005*) does certainly not allow to make
690 detailed statements regarding different levels of visual acuity, though it is sufficient to assess the
691 general visual performance of the mouse strains in question.

692 Physiological Measurements

693 Electroretinography

694 In order to assess the retinal function of male C57BL/6N, CD1, HAB, NAB and LAB animals ($N=6$
695 each), flash-evoked electroretinographic (fERG) measures in the anesthetized animals have been
696 employed. To this end, the animals were dark-adapted for > 3 h prior to the experiment. Under
697 dim red light (650 nm) illumination, the animals were weighed and received analgesic treatment
698 (200 mg/kg Novalgin/Metamizol s.c. in saline in a concentration to obtain $100 \mu\text{l}/10$ g of body
699 weight) and subsequently transferred from their home-cage to the anesthesia chamber (isoflurane
700 4%). After reaching surgical tolerance, indicated by the absence of the eye-lid and paw-withdrawal
701 reflex, the animals were transferred to a modified stereotaxic frame where the anesthesia was
702 maintained with isoflurane (2-3 % in oxygenated air, using an oxygen concentrator, EverFlo). The
703 body temperature was monitored and controlled (37.5°C) using an animal temperature controller
704 (WPI Inc. #ATC2000) in combination with a small rodent rectal temperature probe (WPI Inc. #RET-3)
705 and a small heating-pad (15×10 cm) with built-in RTD sensor (WPI Inc. #61830) with an additional
706 silicone pad to ensure maximal heat transfer (WPI Inc. #503573). For the analgesic treatment to
707 have an effect, the animals was allowed to reach a stable anesthesia for >15 min, while the eyes
708 were kept moisturized with 0.9 % (w/v) physiological sodium chloride solution (saline). Subsequently
709 the pupils were dilated maximally using 2.5 % phenylephrine (Sigma #P6126, in PBS, pH adjusted
710 to 7.0) and 1 % (w/v) atropine (Sigma # A0132, in PBS, pH adjusted to 7.0) and the eyes were
711 henceforward kept moisturized using 1 % methyl cellulose (Carl Roth #8421) in saline. The ERG

712 electrodes were custom made using $\varnothing 200 \mu\text{m}$ uncoated gold wire wound to form $\varnothing 3 \text{ mm}$ loops and
713 were placed gently on the eyes of the animal. A stainless steel wire wrapped around the animal's
714 tail served as the ground electrode. All signals were bandpass filtered at 0.1-300 Hz and sampled at
715 30 kHz using the Open-Ephys (*Siegle et al., 2017*) system in conjunction with a headstage based
716 on the Intan RHD2132 integrated extracellular amplifier circuit. The animals left eye was covered
717 with a piece of light proof black PVC and additionally shielded from the right side using aluminum
718 foil. The animals right side was stimulated using a Ping-Pong ball which was cut in half (*Green*
719 *et al., 1997*) and illuminated with a white LED (Osram Oslon LUW CN7N) which was controlled
720 via a custom-made constant current source. Thereby scotopic and photopic (3 lux background
721 illumination) measurements were carried out which involved the display of 32 light flashes (per
722 condition) of 40-180 μs length at a frequency of 1 Hz at three different light intensities. The light
723 intensities were measured (65 lux, 225 lux, 420 lux) using a hand-held lux meter (Iso-Tech ILM 1335)
724 and the respective $\log \Phi \cdot \text{rod}^{-1} \cdot \text{s}^{-1}$ values were calculated using the following relation:

$$725 \quad 1 \text{ photopic lux} = 650 \text{ photoisomerizations } (\Phi) \cdot \text{rod}^{-1} \cdot \text{s}^{-1} \text{ (Pugh et al., 1998).}$$

726 All 32 acquired responses per condition were averaged and the datasets were further analyzed
727 using custom Python2.7 scripts.

728 Vocalizations

729 During the normal animal care taking procedures, it was realized that HAB mice have a strong
730 disposition to vocalize in the audible hearing range, if lifted at their tails (e.g. at changing cages) and
731 especially when they lose grip from a grid cage top. Although there have been previous attempts to
732 standardize this cage-grid vocalization test (*Whitney, 1970*), in our study the tail-suspension test
733 (TST) was employed, a behavioral test which typically aims to assess depression-like behavior in
734 mice (*Steru et al., 1985*). For this test, the animal was affixed roughly 2 cm above the tail root to
735 a $\varnothing 5 \text{ mm}$ vertical stainless steel rod (20 cm above ground) using heat sterilization tape. Other
736 tapes can be used, but it was found that this sort of material is characterized by its rather low
737 adhesion to murine skin and its excellent removability without introducing skin irritations. The
738 test was carried out within a sound-attenuating chamber. The test duration was 5 minutes, and
739 the animals vocalization was monitored using high-quality sonic/ultrasonic recording equipment
740 (Avisoft UltraSoundGate USG 116-200, condenser microphone CM16/CMPA). Offline analysis was
741 carried out using custom written Python2.7 scripts.

742 Standard Laboratory Procedures & Analysis

743 Stereotaxic Implantation and Virus Injections

744 All stereotaxic surgical procedures were carried out similarly and shall be briefly described. Specifics
745 for cannulae implantations and virus injections are provided if necessary. Before the surgery, the
746 animal was weighed and analgesic treatment (200 mg/kg Vetalgin, Intervet, in saline, s.c.) was
747 administered 15 minutes prior to any other interventions. During this time, all surgical instruments
748 have been heat sterilized and wiped with 70 % ethanol. Then the animal was transferred to the
749 anesthesia induction chamber and slowly anesthetized with isoflurane (0-4 % in oxygenated air,
750 EverFlo Oxygen Concentrator). The absence of the eyelid and paw withdrawal reflex indicated
751 surgical tolerance and the animal was transferred to the stereotaxic frame (Leica Biosystems,
752 AngleTwo), where it was fixed using non-rupture/non-traumatic ear bars and a snout clamp. The
753 anesthesia was kept constant with 2-2.5 % isoflurane, while the animals body temperature was
754 constantly monitored and controlled (37.5°C) using a rodent rectal probe, heating blanket and
755 a animal temperature controller (WPI Inc. ATC2000). The eyes were kept moisturized using eye
756 ointment (Bepanthen® eye and nose ointment). Further the animals head was shaved using either
757 serrated scissors or an electric shaver. Excess cut hair was removed with cotton swabs soaked
758 with lidocaine (Sigma #L7757, 10 % (w/v) in 70 % ethanol) which in addition exerted an additional
759 cutaneous analgesic effect. Using sharp scissor, the skin above the skull was opened from 1 mm

760 caudal to lambda to 2 mm rostral to bregma. The periosteum was removed with clean cotton swabs
761 soaked in lidocaine solution followed by 3 % hydrogen peroxide. Now, using a small and stiff probe
762 the AngleTwo system was calibrated with the position of bregma and lambda and medial-lateral
763 (ML) and dorsoventral (DV) deviations were corrected if necessary to read less than 50 μm utilizing
764 the manufacturing tolerances of the mouse skull adapters' dovetail rails. In order to correct the skull
765 rotation, two contra-lateral coordinates on the skull surface were targeted (ML ± 2.0 mm, AP -1.82
766 mm) and the respective DV coordinates were noted. If a deviation >50 μm was noticed, the ear bars
767 were released and the initial rotation was corrected. Once the position of the skull was sufficiently
768 accurate, implantation or virus injection was conducted. After these procedures the animals were
769 weighed and their general health and healing status was assessed and recorded on a daily basis for
770 5 consecutive days and in addition the animals received post-surgical analgesic treatment (1 mg/kg
771 Metacam, Böhringer Ingelheim, in saline, s.c., daily). For viral injections, a 5 μl Hamilton syringe
772 (7634-01/00) equipped with a blunt 33 gauge needle or a 10 μl WPI Inc. syringe (NANOFIL) equipped
773 with a 34 gauge beveled needle (NF34BV-2) in conjunction with a motorized micropump (WPI Inc.
774 UMP3) and the respective micropump controller (WPI Inc. MICRO4) was used. The injection rate
775 was set to 80 nl/min. For the experiments involving the pharmacogenetic manipulation of the
776 SC in LAB mice, 350 nl of adeno-associated-virus serotype-5 (AAV), expressing either the active
777 DREADD (AAV5-CaMKII α -hM3Dq-mCherry, #AV6333, $N=12$) or just the reporter fluorophore (controls,
778 AAV5-CaMKII α -mCherry, #AV4809c, $N=12$), have been injected (ML ± 0.9 mm, AP -3.64 mm, DV -1.75
779 mm). All viruses were purchased from the Gene Therapy Center Vector Core of the University
780 of North Carolina, Chapel Hill and were diluted, using 350 mM NaCl solution, to reach a target
781 titer of 1.7×10^{12} vg/ml. For the injection, first, the target drilling site was marked with a pencil
782 on the skull surface, and the skull was penetrated using a $\emptyset 0.5$ mm burr with counterclockwise
783 concentric movements until the intact dura mater becomes visible. Using a hypodermic needle,
784 whose foremost sharp tip was gently bent to the outside by tipping it onto a polished stainless
785 steel surface in order to form a micro-miniature hook-like instrument, was used to first remove the
786 remaining skull pieces and secondly to open the dura at the site of injection. The injection needle
787 was slowly lowered to reach the target site and the injection was initiated. After the injection the
788 needle was raised for 100 μm and left for additional 10 minutes in order to allow the virus to diffuse.
789 Subsequently, the needle was removed and the procedure was repeated on the contralateral side.
790 During the injection the wound was kept moisturized using saline, in order to prevent brain tissue
791 from sticking onto the needle and to aid the subsequent cutaneous suture. After the injection,
792 using resorbable, sterile, surgical needled suture material (VetSuture fastPGLA 5/0, 13 mm reverse
793 cutting needle 3/8), the wound was closed with 4-6 intermittent stitches, and treated with iodine
794 solution (BRAUNOL[®]). The incubation time for the virus to reach stable expression was >5 weeks.

795 Guide Cannula Implantation & Local Muscimol Injections

796 For the local injection of muscimol within the IPAG of HAB mice ($N=14$), two 3.0 mm long, 26 gauge
797 guide cannulae (WPI Inc.) have been implanted using an angle of $\pm 25^\circ$ at ML ± 1.02 mm, AP -4.25
798 mm and DV -1.55 mm. As the internal injection needle had a length of 4.0 mm, the ultimate injection
799 site was ML ± 0.6 mm, AP -4.25 mm and DV -2.45 mm. One skull screw per hemisphere above the
800 hippocampus (ML ± 1.5 , AP -1.27) allowed a mechanically stable attachment of the cannulae to
801 the skull using dental cement (Paladur[®], Heraeus-Kulzer). Iodine solution (BRAUNOL[®]) was used to
802 disinfect the wound. After the implantation, dummy injection needles with a dust cap and a length
803 of 3.5 mm were inserted into the guide cannulae in order to prevent clogging. The animals were
804 allowed to recover for more than 2 weeks after the surgery. The injection of MUSC or fMUSC or
805 vehicle (aCSF) before the EPM and vocalization task was conducted in the anesthetized (2-2.5 %
806 isoflurane) animal. The injection was carried out using an ultra micropump (WPI Inc. UMP3) and the
807 injection rate was set to 100 nl/min whereby volume of 100 nl was injected. 45 minutes after the
808 injection, the animals were subjected to the behavioral paradigm.

809 Histology

810 For histological verification of injection and implantation sites, the animals were deeply anesthetized
811 using a mixture of ketamine (50 mg/kg, Essex Pharma GmbH, Germany) and xylazinehydrochloride
812 (5 mg/kg, Rompun, Bayer Health Care, Germany) injected systemically (100 μ l per 10 g body weight,
813 i.p.). Subsequently the animals were given an overdose of isoflurane to induce respiratory arrest
814 (final anesthesia) and transcardially perfused with cold physiological saline followed by 4% (w/v)
815 paraformaldehyde (PFA) in phosphate buffered saline (PBS, final concentrations in mM: 136.89 NaCl,
816 2.68 KCl, 10 Na₂HPO₄, 1.76 KH₂PO₄; pH adjusted to 7.4 using HCl). The brains of the animals were
817 post-fixed in PFA solution for >24 h at 4°C. In order to prevent the implant tracks from collapsing
818 upon removal, the entire heads of the animals were post-fixed for >48 h. The brains were further
819 placed in 30% (w/v) sucrose in PBS solution for >36 h at 4°C for cryoprotection in order to increase
820 tissue rigidity. Subsequently the brains were dry dabbed and carefully frozen by repeatedly dipping
821 the brain, held at the medulla, into the cold 2-methylbutane on dry ice and stored at -80°C. Coronal
822 tissue sections of 35 μ m, cut in several series, were prepared using a cryostat (Thermo Scientific
823 Microm HM560). Sections were collected directly on microscopy slides (SuperFrost®, Menzel-Gläser,
824 Germany). For proteinaceous fluorophores the specimens were covered and preserved using
825 antifade mounting medium (VECTASHIELD® HardSet H-1500, VECTOR Laboratories, UK) containing
826 the nuclear counterstain 4',6-diamidino-2-phenylindole (DAPI). Some series were stained using the
827 standard Nissl staining method in order to reveal the gross anatomical structures. In brief, the
828 specimens were dehydrated using (in v/v) 80%, 90%, 2x100% ethanol (30 seconds per step), stained
829 in 0.1% (w/v) cresyl violet solution in double distilled water acidified with 300 μ l glacial acetic acid
830 for 30 seconds. Subsequently the specimens were differentiated in 100% isopropyl alcohol (for
831 30 seconds) followed by 100% xylene for (>5 min). The cresyl violet stained sections were covered
832 and preserved using DPX mounting medium. For the preparation of retinal section the eyes of
833 the perfused animals were removed and stored in 4% PFA at 4°C and the retinas were extracted.
834 Retinal sections (30 μ m) were obtained (*Ivanova et al., 2013*) using a cryostat and the specimens
835 were stained with haematoxylin and eosin.

836 Genotyping for *Pde6b*^{rd1}

837 The genotyping for *Pde6b*^{rd1} was carried out according to Chang et al. 2013 (*Chang et al., 2013*).
838 In brief, genomic DNA was extracted from tail biopsies (B6, CD1, HAB, NAB, LAB, N=4 per strain)
839 by adding 100 μ l 50 mM NaOH aqueous solution to each sample (per 1.5 mL reaction tube)
840 followed by 30 minutes incubation at 99°C. Subsequently the samples were allowed to cool down
841 and 30 μ l of 1 M Tris-HCl aqueous solution was added per sample. Finally the samples were
842 thoroughly vortexed and cell debris was removed by brief centrifugation and the samples were
843 stored at -20°C. For the polymerase chain reaction (PCR), 2.5 μ l PCR buffer (Thermo Scientific,
844 ThermoPrimeTaq 10x Buffer), 2.5 μ l MgCl₂ (25 mM), 1 μ l deoxynucleoside triphosphate (dNTP,
845 10 mM) mix (Thermo Scientific, 18427-088), 1 μ l dissolved G1 primer, 1 μ l G2 primer, 1 μ l XMV
846 primer, 0.2 μ l Taq DNA polymerase (Thermo Scientific, ThermoPrime, #AB-0301/B) and 14.8 μ l
847 double distilled water was mixed with 1 μ l of genomic DNA solution. The primer sequences were as
848 follows: G1 (5'-CCTGCATGTGAACCCAGTATTCT ATC-3'), G2 (5'-CTACAGCCCCTCTCCAAGGTTATAG-3')
849 and XMV (5'-AAGCTA GCTGCAGTAACGCCATTT-3'). The idea of this three primer design is that while
850 G1 and G2 result in a PCR product of 240 base pairs (bp) from normal non-mutant animals, G2
851 and XMV generate a larger (560 bp) product from the *rd1* mutant allele. The thermal cycler PCR
852 protocol consisted of the following steps: denaturation for 3 minutes at 95°C, followed by 34 cycles
853 of annealing (30 seconds, 55°C) and extension (1 minute, 72°C) terminated with a final cycle at
854 72°C for 5 minutes and the subsequent incubation at 4°C. The amplified DNA was analyzed using
855 agarose gel electrophoresis and a subsequent ethidium bromide staining.

856 Manganese-enhanced MRI

857 The animals (HAB $N=31$, NAB $N=26$, LAB $N=30$) were injected with a low dose of manganese chloride
858 (30 mg/kg in saline, i.p.) for eight consecutive days (8×30/24 h) prior to the scanning procedure, see
859 Grünecker et al. 2010 (*Grünecker et al., 2010*). The MRI experiments were performed in a 7T MRI
860 scanner (Avance Biospec 70/30, Bruker BioSpin, Ettlingen, Germany) at 24 h after the last injection,
861 with the animals being anesthetized with isoflurane (≈ 1.5 -1.7% in oxygenated air). Body tempera-
862 ture was monitored and kept constant in the range 34-36°C. A saddle-shaped receiver coil was used
863 for signal acquisition. T_1 -weighted images were acquired using a 3D gradient echo pulse sequence
864 (repetition time TR = 50 ms, echo time TE = 3.2 ms) using a matrix of 128×128×128 at a field of view
865 of 16×16×18 mm³, yielding a final resolution of 125×125×140.6 μm^3 . 10 averages were acquired.
866 In addition, 3D T_2 -weighted images were acquired using a rapid acquisition relaxation enhanced
867 (RARE) pulse sequence (TR = 1 s, TE = 10 ms) with the same spatial resolution as mentioned above,
868 and two averages. This resulted in a total imaging time of approximately 2 hours per animal. The
869 reconstructed images (Paravision, Bruker BioSpin, Ettlingen, Germany) were further analyzed using
870 the statistical parametric mapping package SPM5 (using the spm mouse toolbox) and SPM8 (using
871 the new segment option for bias correction) (www.fil.ion.ucl.ac.uk/spm/).

872
873 The acquired images of all animals were segmented exploiting mouse specific tissue probability
874 maps, and bias corrected images were obtained. Then, images were spatially normalized in several
875 steps: 1. Normalization of all images (including brain and extracranial tissue) to a representative
876 single animal image and calculation of the mean normalized image. 2. Creation of a brain mask on
877 the mean normalized image. Brain extraction in native space using the back-transformed mean
878 brain mask. 3. Normalization of the brain extracted images to the group template. Finally, images
879 were smoothed using a Gaussian kernel of eight times the image resolution. Data were further
880 analyzed in SPM using a full factorial design with three conditions (HAB, NAB and LAB), global mean
881 correction and global normalization using ANCOVA. A pairwise voxel-based comparison between
882 HAB vs. NAB and LAB vs. NAB (FDR $p<0.001$, cluster extent >20) revealed the differential manganese
883 accumulation ().

884 Statistical Analysis

885 All data are presented as mean values \pm standard error (SEM). Statistical analysis has been per-
886 formed using GraphPad Prism 7. One way analysis of variance (in some cases for repeated
887 measures) was followed by Bonferroni post-hoc analysis. 2-way analysis of variance (ANOVA) for
888 repeated measures (rmANOVA) was followed by Bonferroni post-hoc analysis. Non-parametric
889 analysis was carried out using the Mann-Whitney U test. Contingency tables were analyzed using
890 χ^2 test if the tables were of sufficient size, otherwise the Fisher's exact test was used. A $p<0.05$
891 was considered statistically significant. First, group differences were verified by ANOVA, followed -
892 if appropriate - by post-hoc tests which considered differences between HAB vs. NAB or LAB vs.
893 NAB. As the manifestation of *high-anxiety* and *low-anxiety* phenotypes via selective breeding most
894 likely involved different complex multigenic changes, a direct comparison of HAB against LAB is
895 inappropriate. Therefore we only compared HAB and LAB to the common NAB control.

896 Author Contributions

897 AJG conceptualization, data curation, formal analysis, investigation, methodology, project adminis-
898 tration, software, supervision, visualization, writing - original draft preparation; NA investigation,
899 visualization; SAB investigation; PK formal analysis; DEH investigation; MN resources; ME investiga-
900 tion; SFK methodology, investigation; CJR methodology, investigation; BTB methodology, investiga-
901 tion, visualization; MC resources, software, supervision, visualization, writing - review & editing; CTW
902 formal analysis, methodology, project administration, resources, supervision, validation, writing -
903 original draft preparation, review & editing.

904 **Supporting information**

905 S1 Mov.

906 **Supplemental Movie 1**

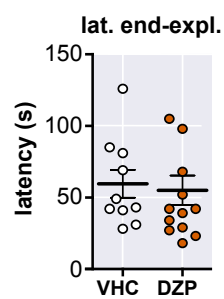
907 Movie explaining the Robocat task also known as the *Panic Box*.

908 S1 Fig.

909 **Supplemental Figure 1**

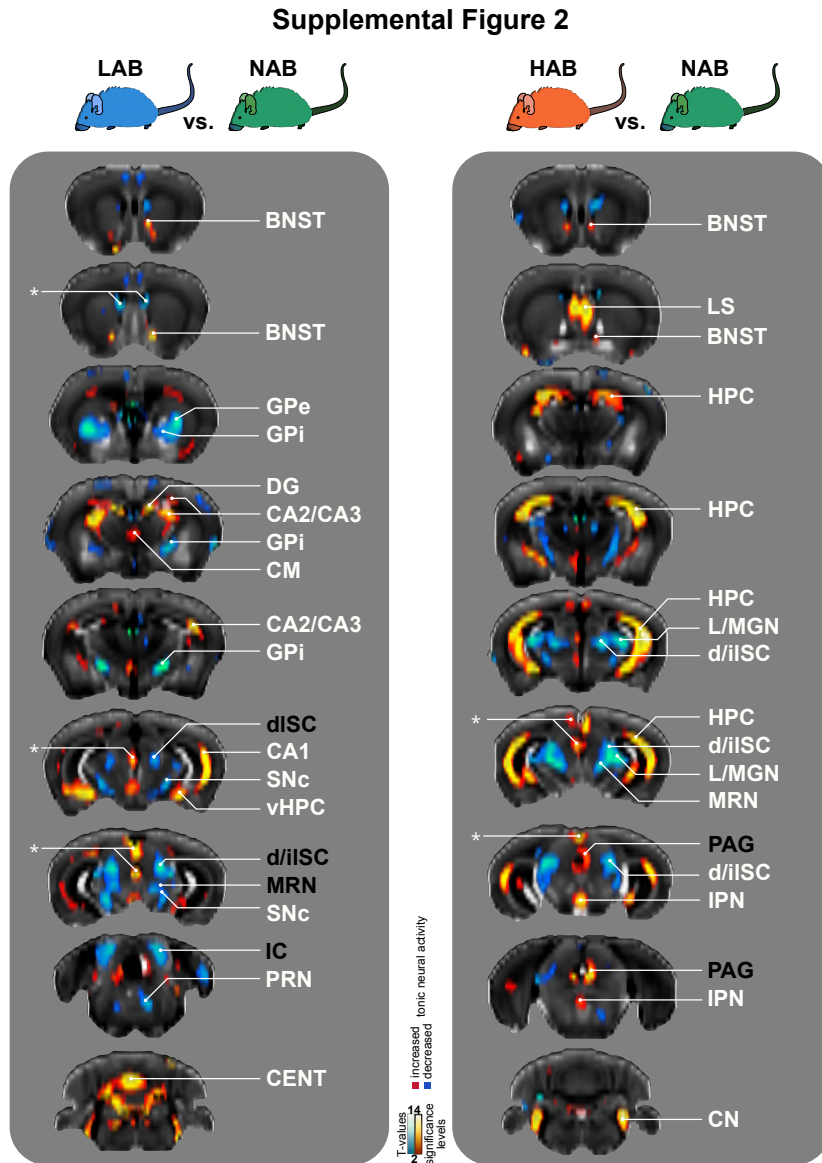
Unaltered latency for end-exploration in the IndyMaze task with DZP treatment.

Supplemental Figure 1



910

911 S2 Fig.
 912 **Supplemental Figure 2**
 Complete MEMRI Data Set.



Asterisks indicate potential artifacts which have been observed to occur close to the brain surface or the ventricular system. **BNST** bed nucleus of the stria terminalis, **CA1-3** cornu ammonis 1-3, **CENT** central lobule of the cerebellum, **CM** central medial nucleus of the thalamus, **CN** cochlear nucleus, **DG** dentate gyrus, **d/iISC** deep/intermediate layers of the superior colliculus, **GPe** globus pallidus external segment, **GPI** globus pallidus internal segment, **HPC** hippocampus proper, **IPN** interpedunclopontine nucleus, **LS** lateral septal nucleus, **L/MGM** lateral/medial geniculate, **MRN** midbrain reticular nucleus, **PAG** periaqueductal gray, **PRN** pontine reticular nucleus, **SNC** substantia nigra pars compacta, **vHPC** ventral portion of the hippocampus proper.

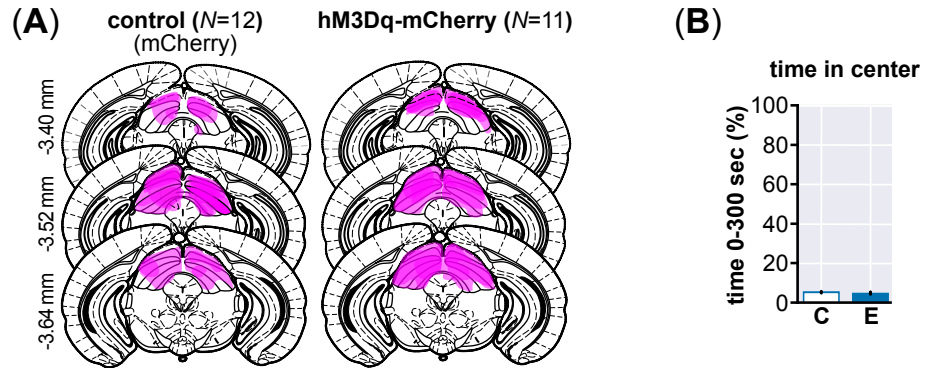
913

914 S3 Fig.

915 **Supplemental Figure 3**

Summary of histological analysis of virus spread in LAB animals and additional EPM measures.

Supplemental Figure 3



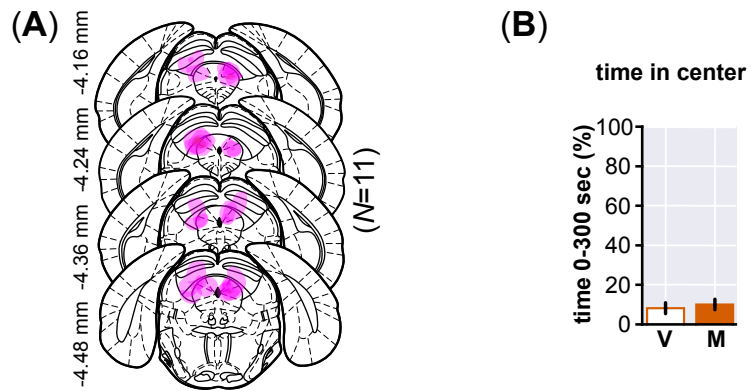
916

917 S4 Fig.

918 **Supplemental Figure 4**

Summary of histological analysis of MUSC spread in HAB animals and additional EPM measures.

Supplemental Figure 4



919

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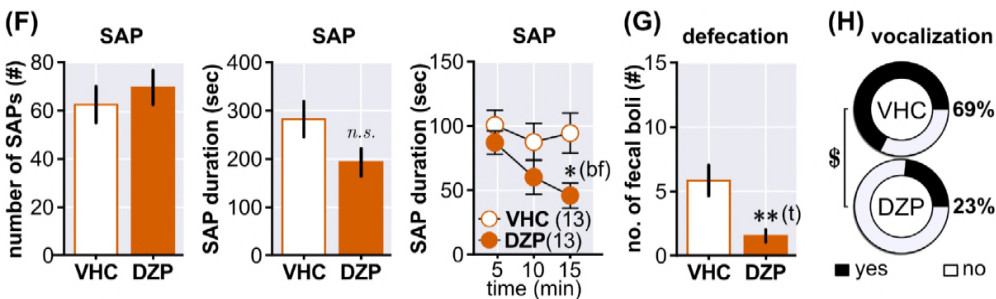
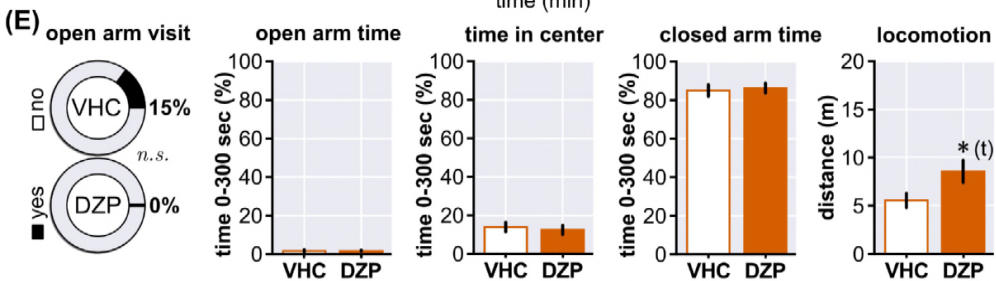
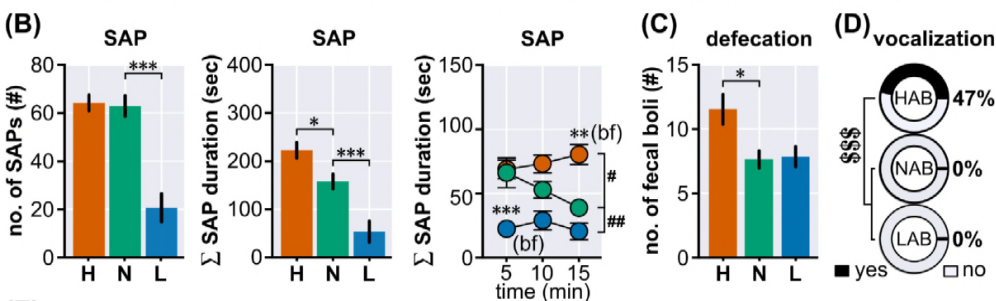
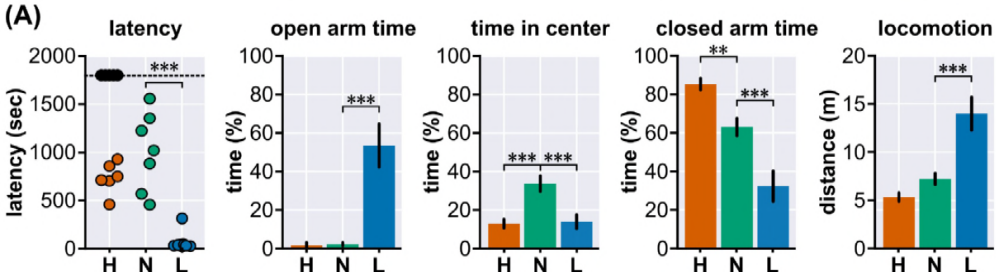
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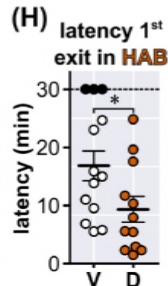
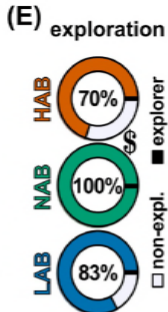
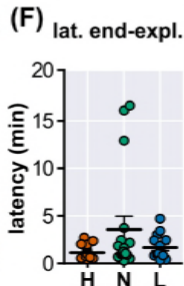
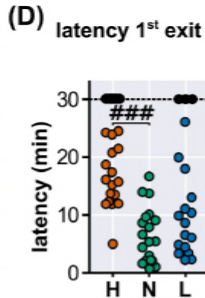
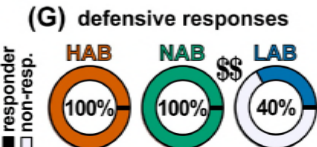
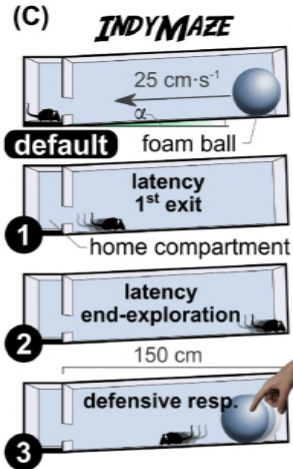
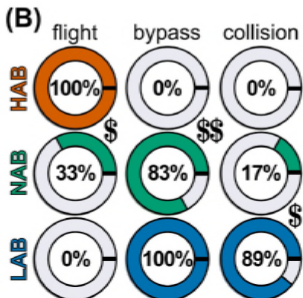
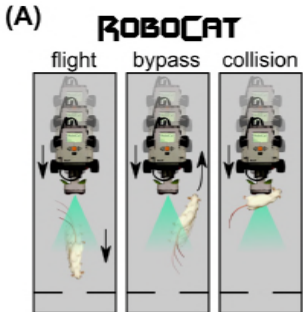
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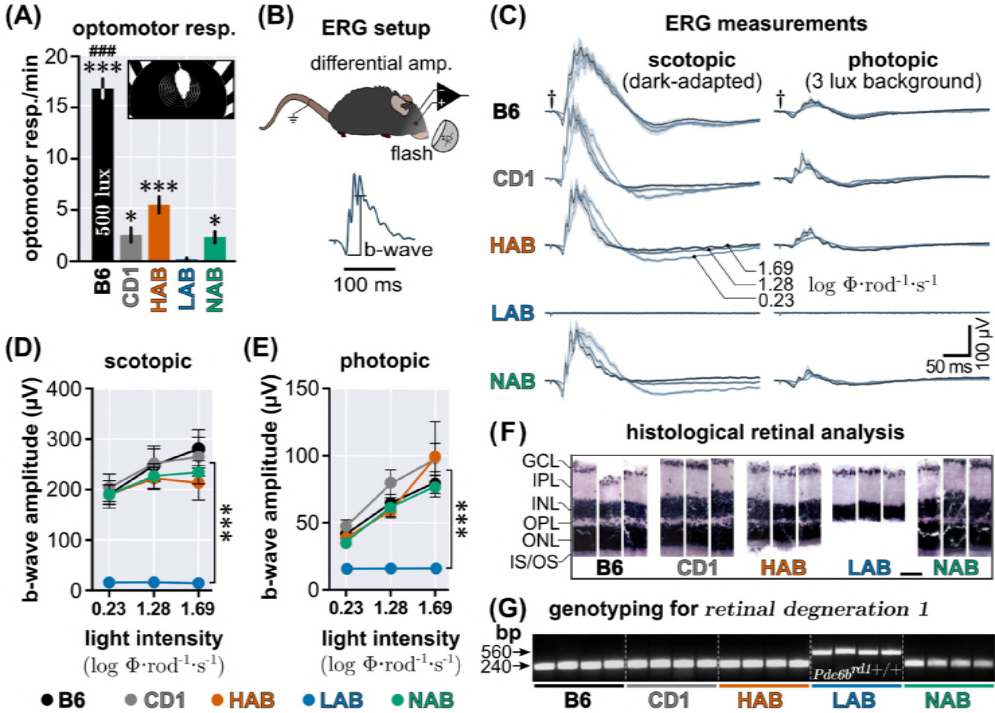
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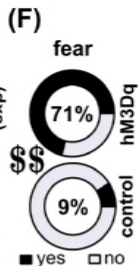
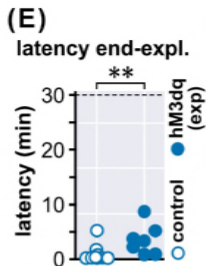
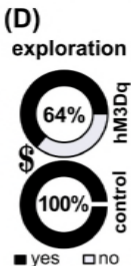
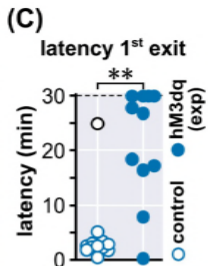
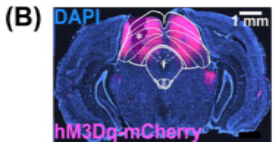
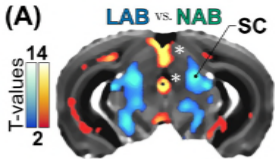
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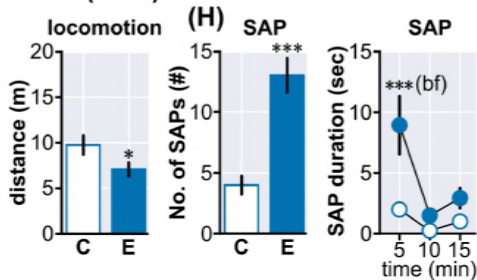
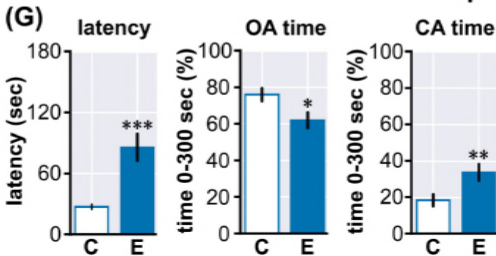


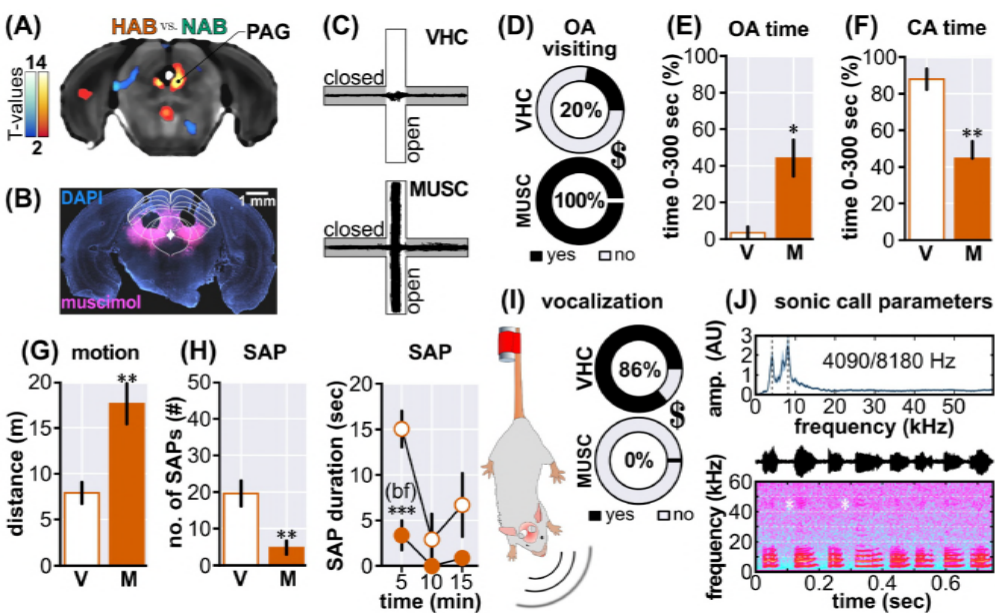




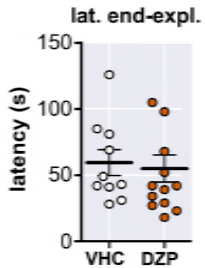


elevated plus maze (EPM)

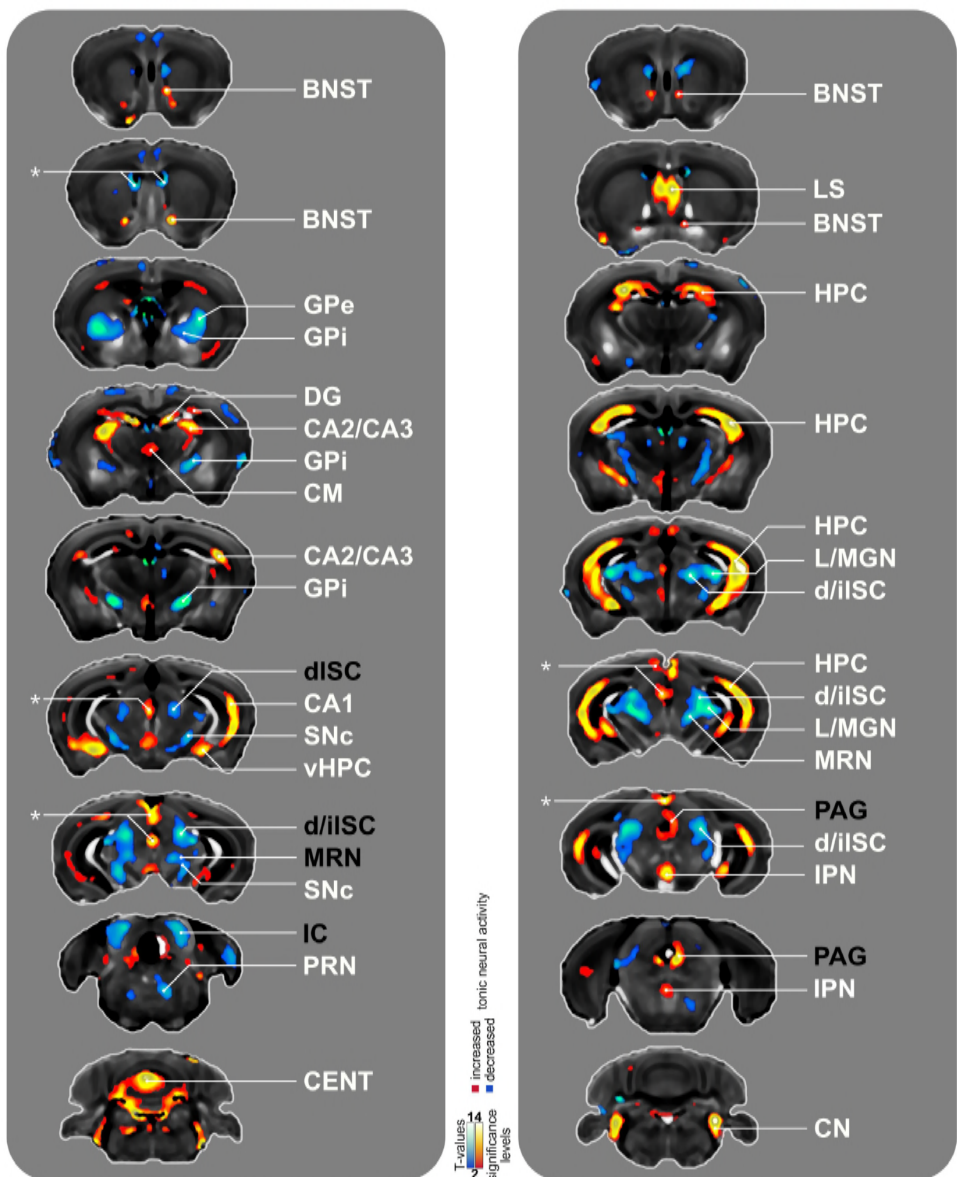




Supplemental Figure 1

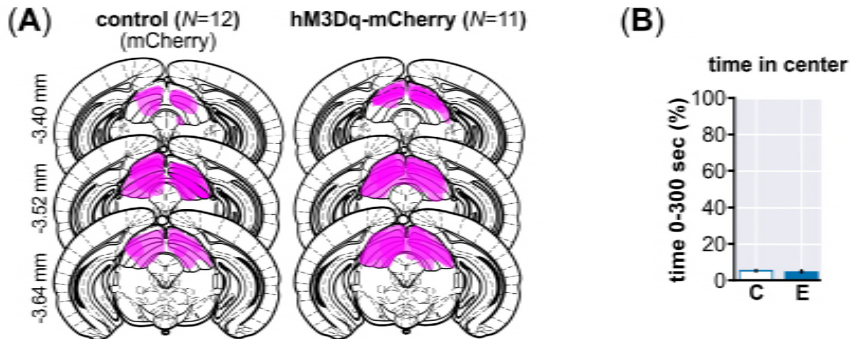


Supplemental Figure 2



Asterisks indicate potential artifacts which have been observed to occur close to the brain surface or the ventricular system. **BNST** bed nucleus of the stria terminalis, **CA1-3** cornu ammonis 1-3, **CENT** central lobule of the cerebellum, **CM** central medial nucleus of the thalamus, **CN** cochlear nucleus, **DG** dentate gyrus, **d/iISC** deep/intermediate layers of the superior colliculus, **GPe** globus pallidus external segment, **GPi** globus pallidus internal segment, **HPC** hippocampus proper, **IPN** interpedunculo-pontine nucleus, **LS** lateral septal nucleus, **L/MGM** lateral/medial geniculate, **MRN** midbrain reticular nucleus, **PAG** periaqueductal gray, **PRN** pontine reticular nucleus, **SNc** substantia nigra pars compacta, **vHPC** ventral portion of the hippocampus proper.

Supplemental Figure 3



Supplemental Figure 4

