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Anxiety-related activity of ventral hippocampal interneurons

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ABSTRACT

Anxiety is an aversive mood reflecting the anticipation of potential threats. The ventral hippocampus (vH) is a key brain region involved in the genesis of anxiety responses. Recent studies have shown that anxiety is mediated by the activation of vH pyramidal neurons targeting various limbic structures. Throughout the cortex, the activity of pyramidal neurons is controlled by GABA–releasing inhibitory interneurons and the GABAergic system represents an important target of anxiolytic drugs. However, how the activity of vH inhibitory interneurons is related to different anxiety behaviours has not been investigated so far. Here, we integrated in vivo electrophysiology with behavioural phenotyping of distinct anxiety exploration behaviours in rats. We showed that pyramidal neurons and interneurons of the vH are selectively active when animals explore specific compartments of the elevated-plus-maze (EPM), an anxiety task for rodents. Moreover, rats with prior goal-related experience exhibited low-anxiety exploratory behaviour and showed a larger trajectory-related activity of vH interneurons during EPM exploration compared to high anxiety rats. Finally, in low anxiety rats, trajectory-related vH interneurons exhibited opposite activity to pyramidal neurons specifically in the open arms (i.e. more anxiogenic) of the EPM. Our results suggest that vH inhibitory micro-circuits could act as critical elements underlying different anxiety states.

1. Introduction

Learning experiences can help us to better cope with anxieties and have been described to induce changes in the human brain volume and connectivity, leading to improved emotional regulation and resilience (Holz et al., 2016; Nechvatal and Lyons, 2013; Santarnecchi et al., 2018). Yet, the neuronal adaptations and mechanisms underlying anxiety coping are poorly understood.

The EPM is a validated behavioural task to study anxiety in animal models (Lister, 1990; Rodgers et al., 1997). The EPM consists of two open arms and two closed arms (i.e. with walls) positioned high above the floor. Rodents, similar to humans, exhibit an innate fear of heights and open spaces (Barnett, 1975; Gibson and Walk, 1960; Poulton and Menzies, 2002). Rodents thus tend to avoid the open arms of the EPM, but not completely, as they are also motivated to explore the environment, for example, for potential food sources or mates (Cryan and Holmes, 2005). When rats or mice are placed on the EPM and left to explore freely, the time spent exploring vs. avoiding open arms can be used as a measure of anxiety and the effects of anxiolytic drugs (Carobrez and Bertoglio, 2005; Cryan and Holmes, 2005; Walf and Frye, 2007). Repeated exposure of rodents to the EPM does not typically lead to substantial changes in anxiety behaviour or preference for the type of arms explored (Andrade et al., 2003; Schrader et al., 2018; Tucker and McCabe, 2017). In our study, we aimed to compare two groups of rats with the same genetic background but with distinct EPM exploration behaviours of the open arms, implying different anxiety levels of these two groups of rats (Hollis et al., 2015; Zalachoras et al., 2022). To achieve this differentiation in the behavioural phenotype, one group of rats was exposed to a goal-directed task additionally to the EPM, while another group of rats was solely exposed to the EPM. Goal-directed behaviour has been suggested to be compromised in anxiety disorders (Alvares et al., 2014; Griffiths et al., 2014; Tull and Gratz, 2008). Moreover, previous goal-related experiences could contribute to the development of mechanisms enabling subjects to better cope with their

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anxiety (Bardi et al., 2012; Lambert et al., 2014). Thus, goal-related training can represent a powerful behavioural tool to mitigate anxiety without directly manipulating neuronal circuits or genes.

Numerous studies have shown that the vH in rodents and its homologue in humans, the anterior hippocampus, are critical for goal-related and emotional behaviours, including anxiety (Bannerman et al., 2004; Fanselow and Dong, 2010; Gulyaeva, 2015; Haghparast et al., 2017; Viard et al., 2011). Rodents and humans with selective lesions of the vH or anterior hippocampus show decreased anxiety when facing potentially threatening contexts (Bach et al., 2014; Bannerman et al., 2003; Kjelstrup et al., 2002). vH neurons that are activated while rodents explore the open arms of the EPM are frequently termed as 'anxiety' neurons. Anxiety neurons are distinct from place cells (Ciocchi et al., 2015), and manipulating their activity modulates neuroendocrine, autonomic or behavioural responses during anxiety (Jimenez et al., 2018; Padilla-Coreano et al., 2016). Anxiety-related information in the vH is selectively routed to the prefrontal cortex, a brain region involved in decision-making, or to the lateral hypothalamus, a region rather implicated in the neuroendocrine response (Ciocchi et al., 2015; Padilla-Coreano et al., 2019). Similarly, goal-related and reward-related information activate subsets of vH projection neurons targeting the nucleus accumbens (Ciocchi et al., 2015; Felix-Ortiz et al., 2013; LeGates

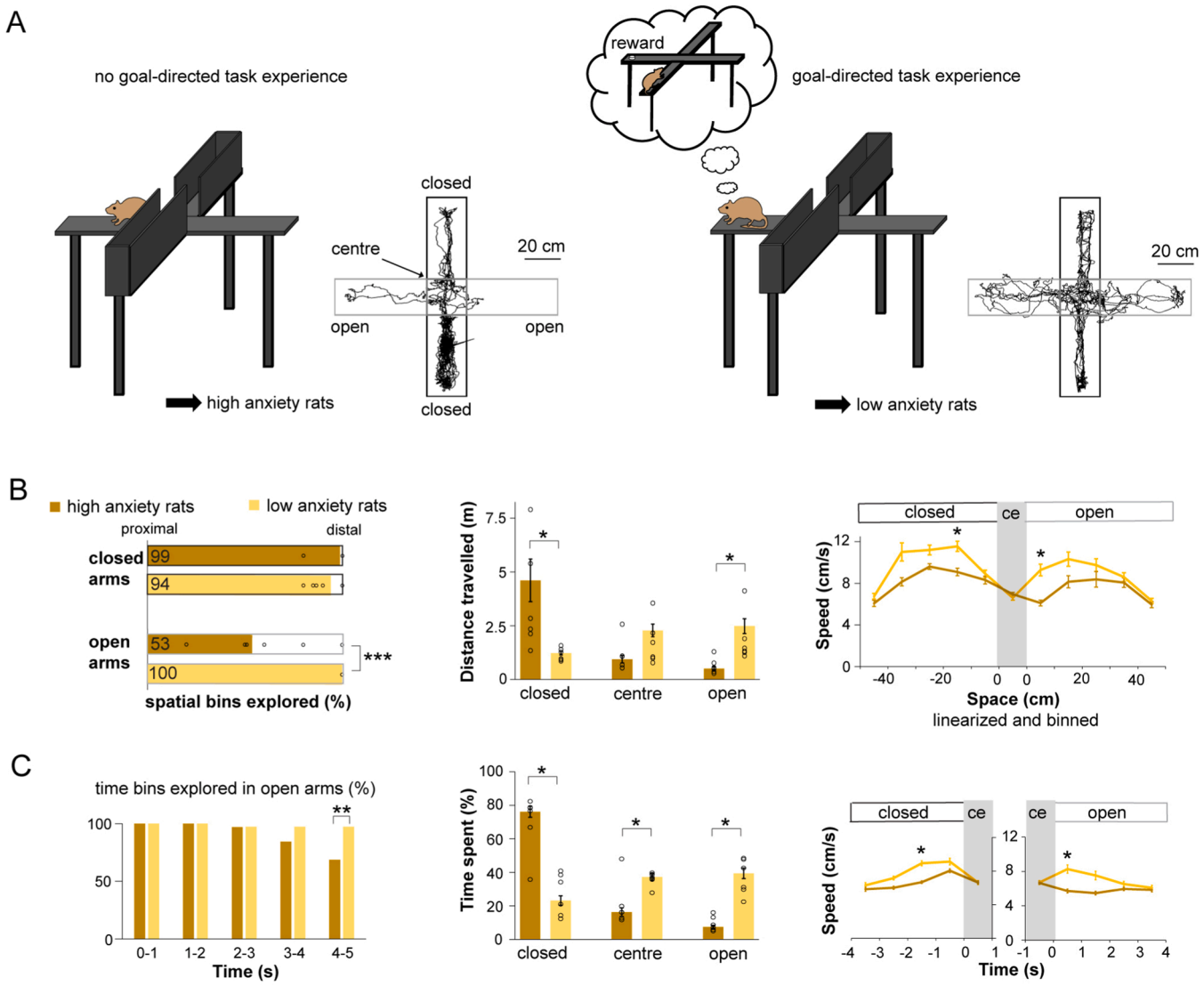

Fig. 1. Goal-related experience increases the exploration of anxiogenic locations. A Schematic of an EPM maze and a top view showing the tracking of a rat. Left, a rat only exposed to the EPM exhibits a low exploration of the open arms of the EPM (i.e. high anxiety rat). Right, a rat also experiencing a reward-based, goal-directed task shows a high exploration of the open arms of the EPM (low anxiety rat). B Differential spatial exploration of the EPM by high anxiety and low anxiety rats. Left, percentage of spatial bins explored in closed and open arms of the EPM. Low anxiety rats explore open arms more distally compared to high anxiety rats (chi-squared test between spatial bins explored in open arms of the EPM in the two rat groups $X^{-2} = 88.1$ and $p < 0.001$). Middle, distance travelled by negative- and low anxiety rats shown in each compartment of the EPM. Low anxiety rats travel more distance in the open arms (and less distance in the closed arms) of the EPM compared to high anxiety rats (two-way ANOVA with significant interaction $F = 27.8$, $p < 0.001$ with post hoc multiple comparisons with $p < 0.05$). Right, low anxiety rats exhibit lower speed (plotted in space) in the closed and open arms of the EPM (shuffling statistics, $p < 0.01$, two-sided, see Methods). Small circles for left and middle panel show average values for each rat. Error bars represent the mean + /- SEM. C Differential temporal exploration of the EPM by high anxiety and low anxiety rats. Left, bar graphs show percentage of time bins (1 sec bins from all sessions) explored with increasing time from the centre. Chi-squared test was only significant for bins 4–5 s between negative- and low anxiety rats ($X^2 = 10.4$ and $p = 0.0012$). Middle, time spent in closed, centre and open compartments of the EPM (two-way ANOVA with post hoc multiple comparisons with $p < 0.05$). Small circles designate average values for individual rats. Right, speed in time in 1 sec bins with reference to the centre (ce). Significant difference between high anxiety and low anxiety rats was determined with shuffling statistics (10000x, significant probability threshold $p < 0.01$, two sided). Error bars represent mean + /- SEM.

et al., 2018; Padilla-Coreano et al., 2016; Pennartz et al., 2011). Given that vH neurons encode both anxiety and goal-related behaviours, this raises the question as to whether goal-related behaviour modulates anxiety behaviour and the underlying vH circuit activity.

Hippocampal neurons are classified into pyramidal neurons, which use glutamate as neurotransmitter and form local and long-distance synaptic connections, and GABAergic interneurons, which control the activity and timing of pyramidal neurons mainly through local inhibitory synapses (Freund and Buzsaki, 1996; Klausberger and Somogyi, 2008). Hippocampal GABAergic interneurons provide feedforward and feedback inhibition onto pyramidal neurons, exhibit synaptic plasticity and impact cell assembly formation during learning (Dupret et al., 2013; Udakis et al., 2020). Furthermore, interneurons of the dorsal hippocampus contribute to working memory, contextual fear conditioning and reward-guided behaviour, and are thus considered to support cognitive and emotional processes (Hu et al., 2014; Lovett-Barron et al., 2014; Murray et al., 2011; Turi et al., 2019). In the context of anxiety, interneurons of the prefrontal cortex expressing the vasoactive intestinal peptide are activated in the open arms of the EPM and gate incoming synaptic inputs arising from the vH, consistent with the role of the vH in controlling anxiety-related activity in the prefrontal cortex (Adhikari et al., 2010; Lee et al., 2019). Yet, the neuronal dynamics of local vH interneurons during the experience of anxiety have not been investigated thus far, even though the vH is critical for the genesis of anxiety responses and the GABAergic system represents an important therapeutic target (Banasr et al., 2006; Hu et al., 2010; Rezvanfard et al., 2009; Zou et al., 2016).

In this study, we generated two groups of rats with different anxiety phenotypes during EPM exploration, while simultaneously recording the activity of vH pyramidal neurons and interneurons. Our results suggest that vH inhibitory micro-circuits may underlie different anxiety states.

2. Results

2.1. Goal-related experience increases the exploration of anxiogenic locations

Two groups of Long Evans rats (n = 6 rats in each group) with different exploration behaviour were tested on the EPM, a validated task to study anxiety in rodents. The first group of rats was solely tested on the EPM while the second group was additionally trained on a reward-based, goal-related task on a fully open plus-maze, before being tested on the EPM (Fig. 1A). In the goal-related task, rats performed trials on a plus maze, in lower light intensity, and of lower elevation, running from a starting arm to a goal arm learning to receive a reward based on egocentric or allocentric strategies (Methods). As a result of this procedure, the group of rats which received the goal-related task training explored the open arms of the regular EPM much further and more extensively in space (Fig. 1B) and time (Fig. 1C) compared to the other group of rats exclusively exposed to the EPM. We termed these two groups of rats with distinct anxiety phenotypes in accordance to previous studies as low anxiety (i.e. with a higher exploration of open arms) and high anxiety rats (i.e. with a lower exploration of open arms), respectively (Hollis et al., 2015; Zalachoras et al., 2022).

While low and high anxiety rats explored similarly the distal extent of the closed arms (Fig. 1B top), only low anxiety rats explored distally the open arms (100% of spatial bins) compared to high anxiety rats (53% of spatial bins explored)(Fig. 1B bottom). Although no significant difference was observed in the number of entries to each type of arms between both rat groups (Supplementary Fig. 1 A), the distance travelled in the different EPM compartments (i.e. closed, centre and open) illustrated the distinct exploration patterns as high anxiety rats covered 76% of the total distance in the closed arms, 15% in the centre, and 8% in the open arms while low anxiety rats covered only 20% of the distance in closed arms, but 38% in the centre and 41% in the open arms (Fig. 1B, middle). The speed of exploration between the two groups of rats also differed, with high anxiety rats moving slower proximally to the centre compared to low anxiety rats (Fig. 1B, right). Importantly, low anxiety rats likely processed anxiety-related information as they exhibited reduced speed when exploring the open arms compared to closed arms of the EPM (Supplementary Fig. 1B). The analyses in the time domain equally highlighted distinct but persistent exploratory features between high anxiety and low anxiety rats (Fig. 1C, Supplementary Fig. 1 C). Collectively, these results suggest that the prior experience of a goal-related task differentiated rats, with a same genetic background, into two groups with distinct anxiety behaviours.

2.2. Identification of pyramidal neurons and interneurons in vH

To identify the neuronal correlates of distinct anxiety-level behaviours during EPM exploration, we chronically recorded extracellular spikes from vH with tetrodes (Supplementary Fig. 2 A, $n = 53$ recording sessions for the two groups of rats). Consistent with previous studies, spikes were isolated into single-units using a semi-automatic clustering and classified into putative pyramidal neurons and interneurons using spike width, spike asymmetry, burst-firing and firing rates (Csicsvari et al., 1998; Sirota et al., 2008)(see Methods). Accordingly, the recorded single-units with high firing rates (i.e. more than 20 Hz) correlated with narrower spike widths (Supplementary Fig. 2B-D). These high-firing, narrow-spikes neurons, exhibited characteristics of interneurons. Additionally, they demonstrated more symmetrical spike shapes compared to their low-firing, wide-spikes pyramidal neurons counterparts (Sirota et al., 2008)(Supplementary Fig. 2E-G, see Methods). Hence, using these parameters, we categorized a total of 426 putative vH pyramidal neurons and 224 putative interneurons out of 852 initially clustered units (Fig. 2A, Supplementary 2 F).

The activity of pyramidal neurons and most notably interneurons in the dorsal hippocampus has been described to be modulated by changes in speed (Czurko et al., 2011; Geisler et al., 2007; Gois and Tort, 2018). Moreover, during EPM exploration, rats move with highly variable speeds and high anxiety and low anxiety rats demonstrate significant speed differences (Fig. 1B,C). Therefore, to examine the neuronal activity in vH pyramidal neurons and interneurons during EPM exploration independent of speed influences, we fitted a general linearized model (GLM) using speed as an explanatory variable (Supplementary Fig. 3) (see Methods). For neurons with significantly correlated activity with speed, the residual neuronal activity from the GLM (i.e. the neuronal activity not explainable by variations in speed) was used in subsequent analyses.

2.3. Selective activation of vH pyramidal neurons and interneurons in different compartments of the EPM

Previous work has shown that the activity of vH pyramidal neurons is selectively elevated when animals explore specific compartments of the EPM (Ciocchi et al., 2015; Jimenez et al., 2018). Pyramidal neurons firing in the open arms of the EPM (classically termed as 'anxiety neurons') are considered to underlie the experience of anxiety, whereas recruitment to the centre or the closed arms is thought to reflect the neuronal correlate of approach-avoidance or safety behaviour, respectively (Adhikari et al., 2011). First, we confirmed the activation of vH pyramidal neurons in distinct compartments of the EPM as previously reported (Fig. 2B). The GABAergic system represents an important target of anxiolytic drugs (Nuss, 2015), however, whether vH interneurons are activated during anxiety behaviour has remained elusive. Our experiments revealed that vH interneurons– similar to vH pyramidal neurons– also exhibit compartment-specific recruitment by being activated when animals were in distinct types of EPM arms (open arms or closed arms) or in the centre of the EPM (Fig. 2B,C,D). Moreover, a higher percentage of vH interneurons were activated in EPM compartments compared to pyramidal neurons (Fig. 2E, 51% of vH interneurons vs 30% of vH pyramidal neurons activated). Yet, the proportions of vH pyramidal

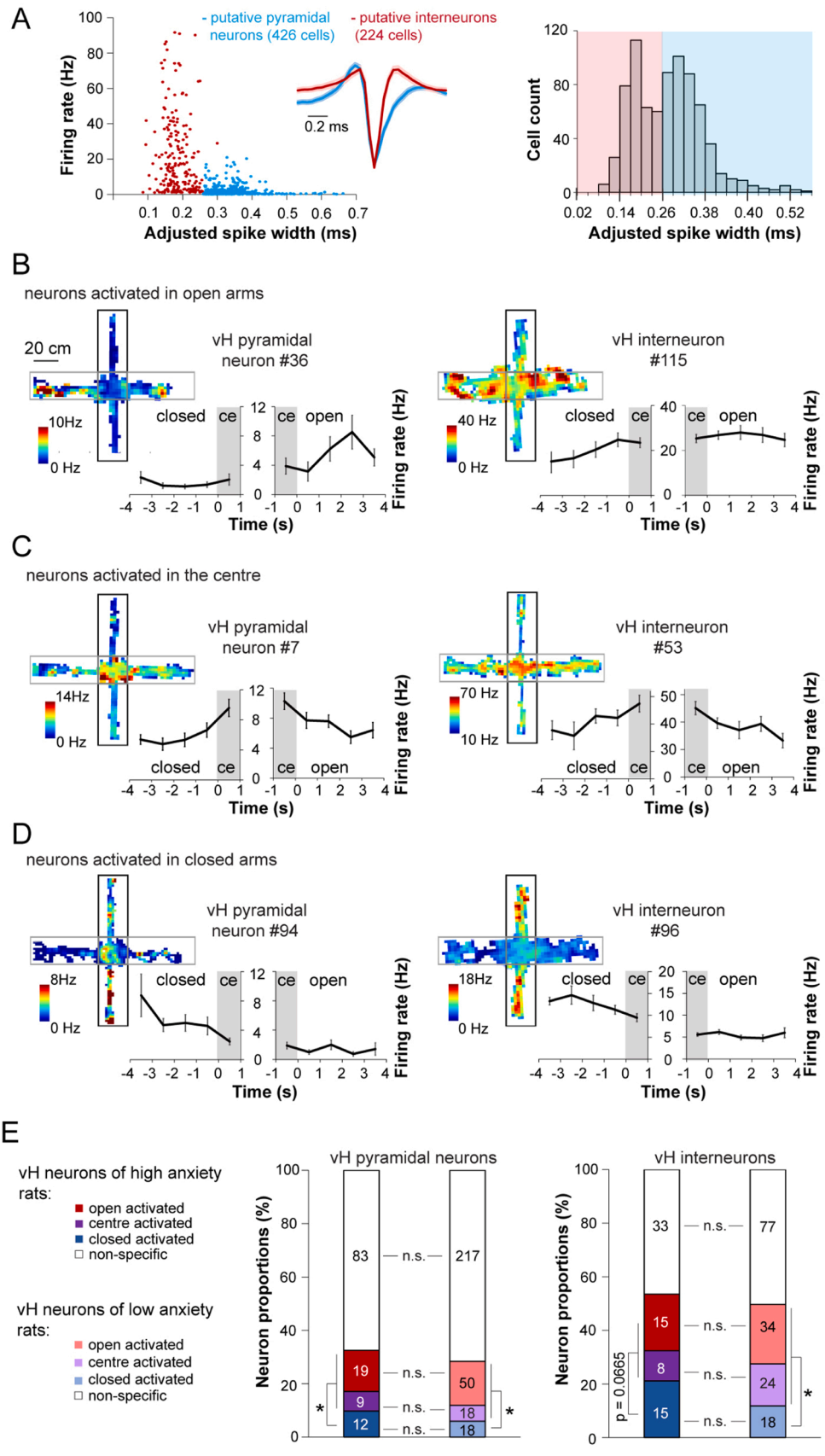

Fig. 2. Selective activation of vH pyramidal neurons and interneurons in different compartments of the EPM. A Left, firing rates over adjusted spike widths of classified putative pyramidal cells and putative interneurons. Middle, average normalized spike shapes of all pyramidal cells and interneurons, shaded lines representing 98% confidence intervals. Right, bimodal distribution of cell counts across different adjusted spike widths (Methods). Background shading designates classification (blue: pyramidal cells and red: interneurons). B Spatial heatmaps and time plots of significant pyramidal neurons and interneurons activated in the open arms of the EPM. Time bins used for statistical testing: 4 sec into arms and 2 × 1 sec in centre. Mann-Whitney U test, with bonferroni corrected alpha = 0.0167, for pyramidal neuron: $Z = 5.3$ and $p < 0.001$, interneuron: $Z = 3.89$ and $= p < 0.001$. Error bars represent mean + /- SEM. C Spatial heatmaps and time plots of significant pyramidal neurons and interneurons activated in the centre of the EPM. Statistical tests as in B. Pyramidal neuron: $Z = 3.2$ and $p = 0.001$, interneuron: $Z = 2.57$ and $p = 0.00988$. Error bars represent mean + /- SEM. D Spatial heatmaps and time plots of significant pyramidal neurons and interneurons activated in the closed arms of the EPM. Statistical tests as in b. Pyramidal neuron: $Z = -6.8$ and $p < 0.001$, interneuron: $Z = -10.21$ and $p < 0.001$. Error bars represent mean + /- SEM. E Similar proportions of compartment activated cells across high anxiety and low anxiety rats (Chi-squared test, not significant (n.s.) with $p > 0.05$). Neurons activated by open spaces are overrepresented. Chi-squared test for high anxiety and low anxiety pyramidal neurons and low anxiety interneurons with $p < 0.001$, Chi-squared test for high anxiety interneurons $X^2 = 3.37$ and $p = 0.0665$. Number of compartment activated pyramidal neurons compared to compartment activated interneurons, Chi-squared test, $X^2 = 28.64$ and $p < 0.001$. Numbers within bars show cell counts.

neurons and interneurons activated in specific EPM compartments did not differ between high anxiety and low anxiety rats (Fig. 2E). Together, these data suggest that both vH pyramidal neurons and interneurons represent different compartments of the EPM imbued with distinct emotional contents.

2.4. Differential representation of open arms in high anxiety and low anxiety rats

Following-up we investigated the time point where the firing of EPM-responsive vH neurons changed, as the animal transits from the safe to the anxiogenic parts of the EPM. We examined differences in neuronal activity in the time domain between high anxiety and low anxiety rats. We found that–among the different categories of vH pyramidal neurons–only vH pyramidal neurons activated in the open arms of the EPM (i.e. anxiety-related vH pyramidal neurons) exhibited higher activity in high anxiety rats compared to low anxiety rats (Fig. 3A). Interestingly, this differential activity between high anxiety and low anxiety rats was observed as rats made transitions between the centre and the open arms of the EPM. The firing of vH pyramidal neurons activated either in the centre or in the closed arms was not different between the two groups of rats. Similarly, vH interneurons activated in the open arms or the centre did not differ between high anxiety and low anxiety rats (Fig. 3A,B). However, closed arm activated vH interneurons exhibited a divergent activity between the two groups of rats during open arm exploration. Specifically, vH interneurons in low anxiety rats displayed an increase in their firing rate during transitions between the centre and the open arms of the EPM (Fig. 3C). Collectively, these data suggest that differences in anxiety-coping behaviour are reflected in selective populations of vH pyramidal neurons and interneurons, with possible (dis-)inhibitory interactions between task-responsive neurons.

2.5. Trajectory-related activity of vH interneurons is predominant and stable in low anxiety rats

Our analyses of vH neurons in high anxiety and low anxiety rats have revealed EPM-compartment-selective neuronal activity, but this approach does not fully capture the multiplicity of EPM exploration patterns. Moreover, animals may exhibit trajectory-related activity on the EPM when venturing to open arms or back to closed arms to reach a safer location. We therefore developed a trajectory-based analysis accounting for different paths taken by rats during EPM exploration to test whether the previous training to a goal-related task (Fig. 1) could lead to a selective modulation of neuronal activity in vH subpopulations during trajectories associated with different anxiety levels. We defined four EPM trajectory-types based on rats either moving from open-arms to the centre to closed-arms, closed-arms to centre to open-arms, open-arms to the centre to open-arms and closed-arms to the centre to closed-arms

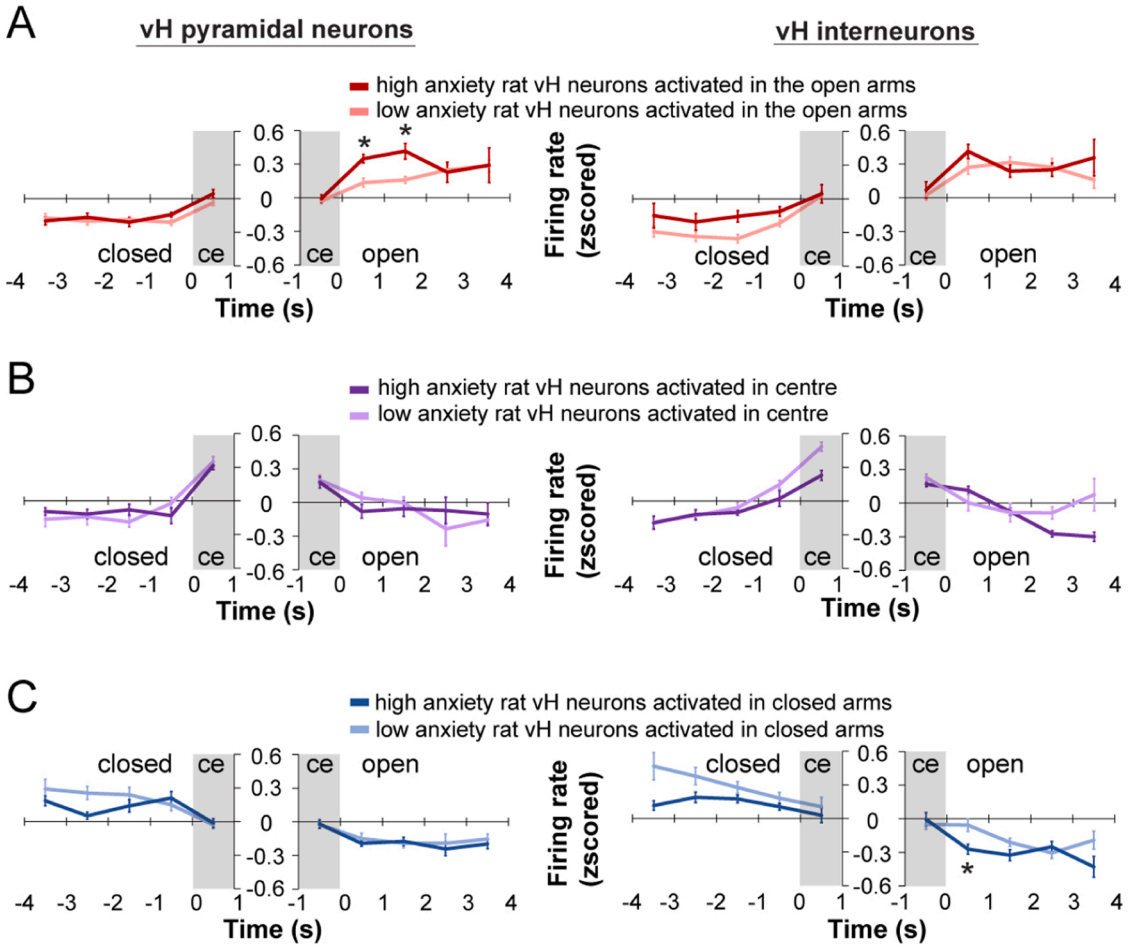

Fig. 3. Differential representation of open arms in high anxiety and low anxiety rats. A Time-dependent firing rates (zscored) of anxiety-related/open activated pyramidal neurons and open arm activated interneurons. On the left panel, vH pyramidal neurons and on the right panel, vH interneurons. Darker colours show activities of high anxiety rats and lighter colours activities or low anxiety rats. Significant differential firing of pyramidal cells in the open arms were determined with shuffling statistics, 10000x, two sided, $p < 0.01$). Error bars represent mean + /- SEM. B Time-dependent firing rates of centre compartment-recruited neurons as in A. C Time-dependent firing rates of closed compartment-recruited neurons as in A. Significant differential firing of interneurons in the closed arms were determined with shuffling statistics, 10000x, two sided, $p < 0.01$). Error bars represent mean + /- SEM.

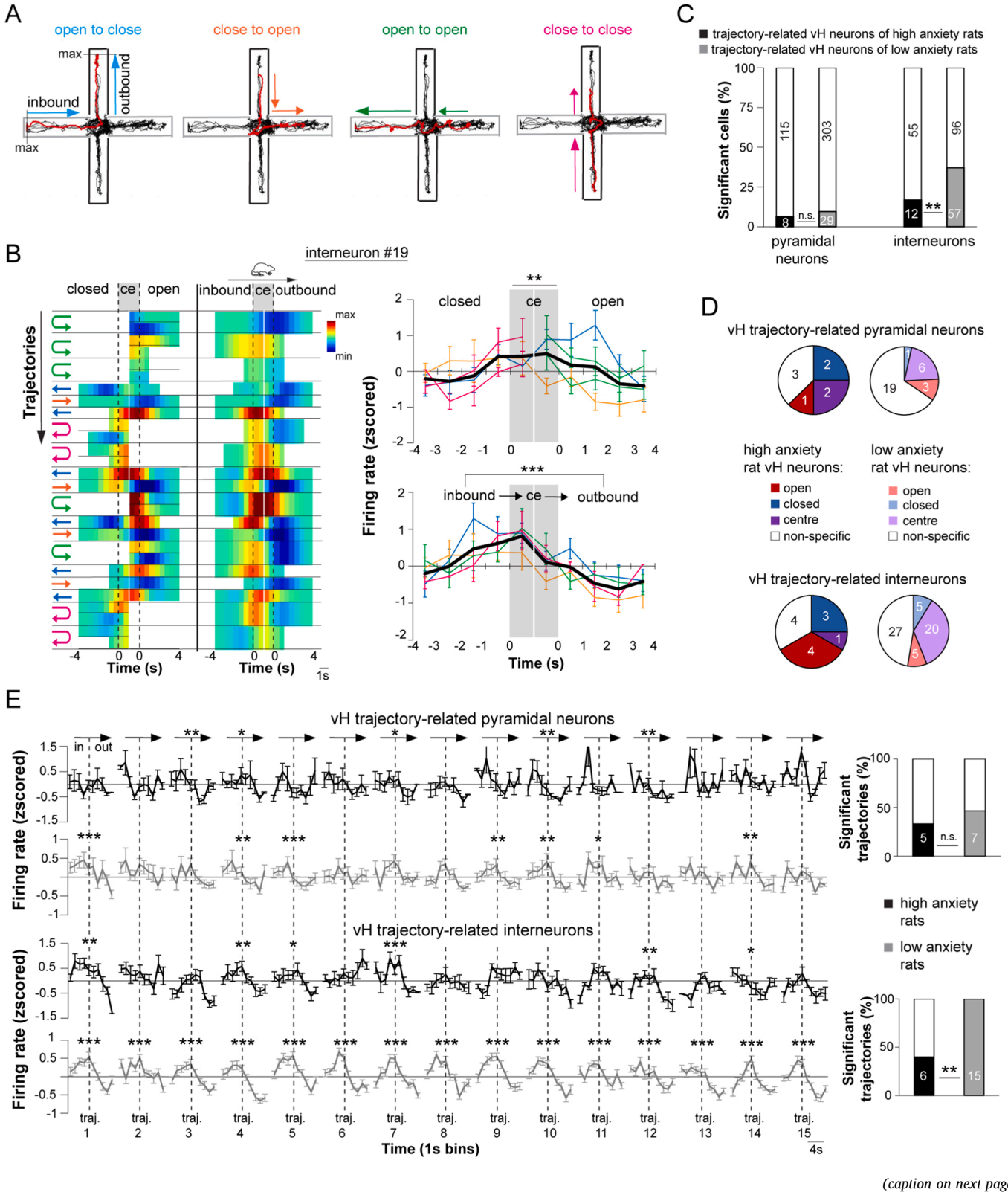

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Fig. 4. Trajectory-related modulation of activity of vH interneurons is predominant and stable in low anxiety rats. A Highlighted trajectory examples (in red) of four defined trajectory types observed on the EPM (black trajectories show the complete trajectories of the session). A trajectory was defined going from maximum (= max., furthest) arm position to the centre and then proceeding to the maximum position of another arm visit (same or different arm). Inbound refers to part of the trajectory going from the maximum position on arm towards the centre and outbound refers to going from the centre to another maximum arm position. Arrows depict the direction of movement. B Left, Activity of an interneuron recruited to the centre of the EPM, trial by trial arranged from close to open (left heatmap) and rearranged from inbound to outbound trajectories (right heatmap). Right, averaged time-dependent and z-scored activities with colours corresponding to trajectory types shown in A and overall mean in black. Mann-Whitney U test was used to determine significant centre activation: $Z = 3.08$ and $p * * = 0.0021$, and inbound to outbound significant decrease, $Z = 5.3$ and $p * ** < 0.001$. Error bars represent mean + /- SEM. C Proportions of significant trajectory-related neurons with decreased activity on the outbound trajectories. Numbers within bars show number of neurons. Neurons from high anxiety rats are compared with low anxiety rats with a Chi-square test (pyramidal neurons with $p > 0.05$ not significant (n.s.) and interneurons $X^2 = 9.43$ and $p = 0.0021$). D Mixed selectivity of trajectory-related neurons. Neuron counts of trajectory-related neurons also tuned to specific EPM compartments (as in Fig. 2) are shown for pyramidal neurons and interneurons of high anxiety and low anxiety rats. E Differential population activity (zscored) across trajectories and analysed trajectory by trajectory (traj.) from inbound (in) to outbound (out) of the trajectory-modulated neurons in C. Significant trajectory-related decrease of activity is marked by stars and most prominent in low anxiety interneurons (paired Wilcoxon test, $* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$). Right, significant difference between trajectories of interneurons from high anxiety and low anxiety rats but not among pyramidal cells as shown in the bar diagrams (Chi-squared test $X^2 = 10.16$ and $p = 0.0014$). Error bars represent mean + /- SEM.

(Fig. 4A, see Methods). We hypothesized that trajectory-dependent navigation implied some goals to reach EPM locations (centre and extremities of the EPM), where decisions are taken to avoid or approach new arms (i.e. vicarious trial and error behaviour)(Redish, 2016) and subsequently restart new trajectories. Our analysis of how much time rats spent in different locations of the EPM during trajectory-dependent behaviour showed that rats of both groups spent more time at the periphery and particularly at the centre of the EPM compared to the centre of the arms. Furthermore, low anxiety rats showed even greater time spent at the centre of the EPM, suggesting that this is an important goal and decision-related location (Supplementary Fig. 4). Thus, trajectory-dependent navigation on the EPM did not merely represent a random or uniform spatial exploration of the EPM.

Using these different trajectories, we discovered a strong modulation of neuronal activity during EPM exploration: First, in the form of a ramping-up activity as animals moved towards the centre (corresponding to an inbound trajectory) and, second, with a ramping-down activity as rats moved away from the centre (corresponding to an outbound trajectory). As illustrated in Fig. 4B, resorting the activity in the closed arms, centre and open arms of a representative vH interneuron into inbound-to-outbound trajectories resulted in a prominent decrease of activity in all of the outbound trajectories (Fig. 4B), an effect also manifesting among different populations of vH pyramidal neurons and interneurons (Supplementary Fig. 5). In stark contrast, very few vH neurons (pyramidal neurons: 2.6%, interneurons: 4.9%) showed an increase in activity during inbound-to-outbound navigation on the EPM (Supplementary Fig. 6). We termed neurons exhibiting the previously described significant decrease of activity as 'trajectory-related neurons'. A comparison between high anxiety and low anxiety rats revealed that the trajectory-related activity was differentially represented among vH pyramidal neurons and interneurons. There was no difference between trajectory-related pyramidal neurons of high anxiety and low anxiety rats (6.5% vs. 9.5%) whereas low anxiety rats exhibited a larger number of trajectory-related vH interneurons compared to high anxiety rats (37.2% vs. 16.9% respectively)(Fig. 4C). Notably, neurons exhibiting trajectory-related modulation did substantially overlap with the different EPM-compartment-activated neurons, suggesting a mixed selectivity of trajectory- and anxiety-related activity in the vH (Fig. 4D). Examining trajectory-related activity over trials across neuronal populations and rat groups indicated that this activity pattern did not develop over time by accumulating trajectories or due to a continuous decrease in overall activity over trajectories. Instead, in trajectory-related vH interneurons of low anxiety rats (e.g. the neuronal population with the largest trajectory-related activity), we rather observe a 'reset' in ramping activity for each trial with a stable sequence of ramping-up (i.e. in inbound trajectories) and ramping-down (in outbound trajectories) activity (Fig. 4E). Together, our results show that trajectory-dependent modulation of vH interneuron activity is predominant and stable in low anxiety rats.

2.6. Trajectory-related vH interneurons interact with pyramidal neurons during anxiety

What might be the effect of the selective trajectory-related activity of vH interneurons on pyramidal neurons during EPM exploration in the low anxiety rats? We hypothesized that trajectory-related vH interneurons may contribute to a differential activity of anxiety-related vH pyramidal neurons (i.e. activated in the open arms of the EPM) in high anxiety vs. low anxiety rats. As we revealed a trajectory-related activity through trajectory-dependent analysis, we therefore examined the activity patterns of anxiety-related pyramidal neurons from the two rat groups during inbound and outbound trajectories in the open and closed compartments of the EPM (Fig. 5). We found that the anxiety-related pyramidal neurons in high anxiety rats were more active compared to low anxiety rats in specific epochs during the exploration of the open arms, but were overall not trajectory-related (Fig. 5B). In contrast, trajectory-related interneurons in low anxiety rats but not high anxiety rats were strongly trajectory-related in open arms with a differential activity during inbound trajectories particularly before entering the centre (Fig. 5B). Though the averaged activity of anxiety-related pyramidal neurons was not trajectory-related, additional trajectory analysis suggested that the activity of anxiety-related pyramidal neurons in low anxiety rats was reduced during inbound trajectories during the same time epochs when the greatest differential activity of the interneurons was observed (Supplementary Fig. 7). To further support the conjecture of an interaction between trajectory-related vH interneurons and anxiety-related pyramidal neurons in the open arms of the EPM, we performed a temporal correlation of their activity in inbound and outbound trajectories in high anxiety and low anxiety rats. We found that trajectory-related vH interneurons and anxiety-related pyramidal neurons were significantly and negatively correlated in low anxiety but not in high anxiety rats (Fig. 5C, Supplementary Fig. 8). Interestingly, when examining the activity in the closed arms of the EPM for these same neuronal populations, there was an absence of differential activity in anxiety-related vH pyramidal neurons and trajectory-related interneurons between low anxiety and high anxiety rats (Fig. 5E). In contrast to the open arms, the same populations of pyramidal neurons and interneurons both expressed trajectory-related activity, and their firing was positively correlated in the two groups of rats (Fig. 5F). Monosynaptic interactions between spikes of trajectory-related vH interneurons and anxiety-related pyramidal neurons exposed 28 out of 61 possible pairs with putative inhibitory interactions in the open arms of low anxiety rats, compared to 9 out of 24 possible pairs in high anxiety rats (Supplementary Fig. 9 A, B). In line with the correlation analyses, this inhibition in the open arms was greater in low anxiety rats than high anxiety rats (Supplementary Fig. 9 A, B, C, D). In the closed arms, the same pairs of neurons, in low anxiety rats, exhibited weaker inhibitory interactions but rather a pattern of co-activation (Supplementary Fig. 9 A, B). This could suggest different functional interactions between

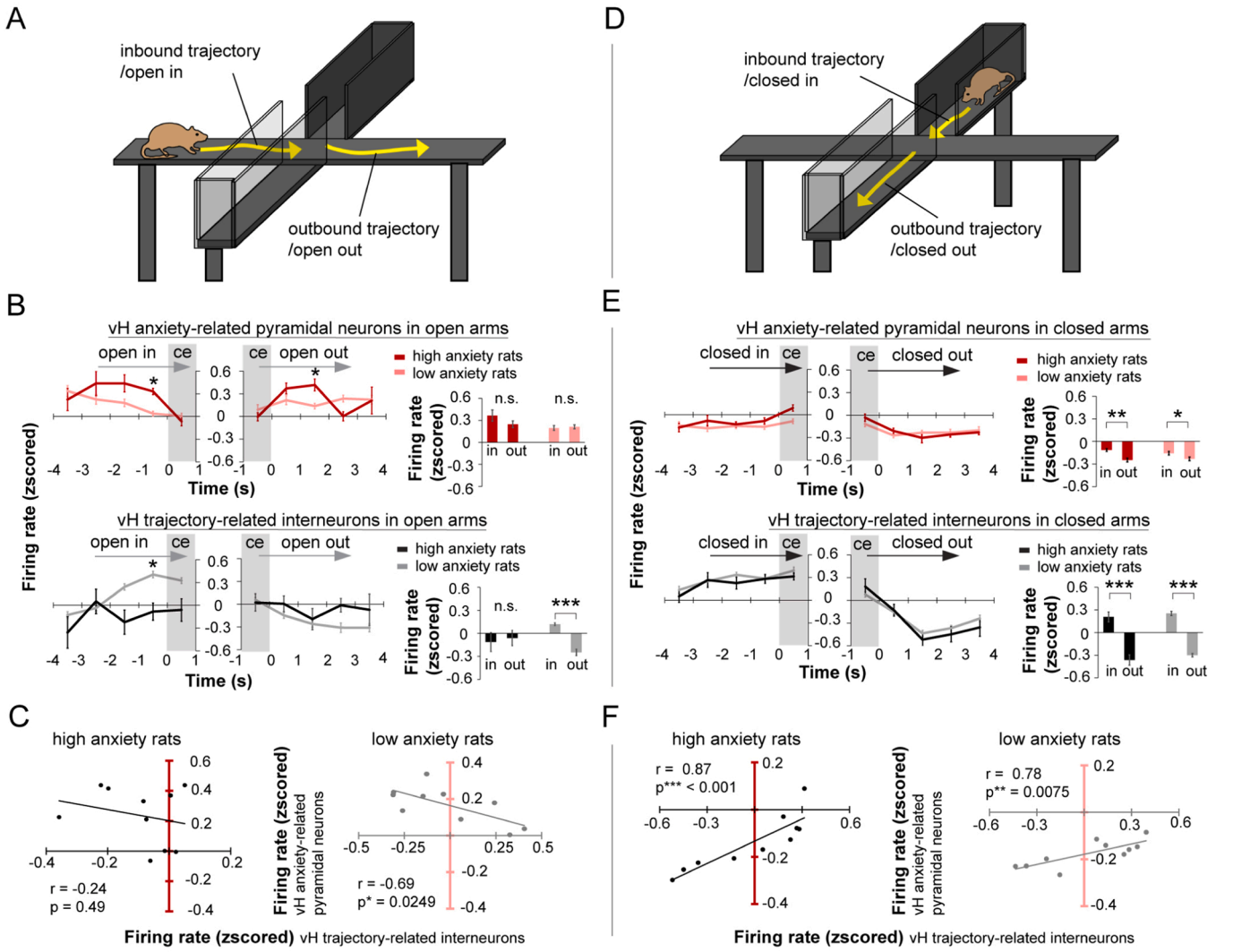

Fig. 5. Trajectory-related vH interneurons interact with vH pyramidal neurons during anxiety. A Schematic of EPM maze with inbound and outbound trajectories solely in the open arms of the EPM. Yellow arrows denote the inbound and outbound trajectories compromised of 4 s in the open arms and up to 1 s after entering or before leaving the centre. B Top, differential activity of anxiety-related pyramidal neurons between high anxiety and low anxiety rats during inbound and outbound trajectories in the open arms (shuffling statistics 10000x, significant probability threshold $p < 0.01$, two sided). Non-significant (n.s.) trajectory-related activity of pyramidal cells shown in the bar diagrams. Bottom, differential activity of trajectory-related interneurons between high anxiety and low anxiety rats during inbound and outbound trajectories in the open arms (shuffling statistics 10000x, significant probability threshold $p < 0.01$, two sided). Significant trajectory-related decrease of interneuron activity shown in the bar diagrams (paired Wilcoxon test, $Z = 5.32$ *** $= p < 0.001$). Error bars represent mean + /- SEM. C Inhibition of anxiety-related pyramidal neurons by goal-related interneurons in low anxiety rats suggested by a significant negative correlation, absent in high anxiety rats (right and left respectively, Pearson's correlation). Pyramidal population activity plotted over interneuron population activity (colours of the axes correspond to neurons as defined in B). D Schematic of EPM maze with inbound and outbound trajectories solely in the closed arms of the EPM. Yellow arrows denote the inbound and outbound trajectories compromised of 4 s in the closed arms and up to 1 sond after entering or before leaving the centre. E Top, no difference between high anxiety and low anxiety rats of the same pyramidal and interneuron population as in b (shuffling statistics 10000x, $p > 0.01$). Significant trajectory-related decrease of activity of pyramidal neurons and interneurons shown in the bar diagrams to the right (paired Wilcoxon test, pyramidal neurons of high anxiety rats inbound to outbound $Z = 2.72$ and $p = 0.0065$, pyramidal neurons of low anxiety inbound to outbound $Z = 2.34$ and $p = 0.0192$, goal-related interneurons of high anxiety rats $p < 0.001$, trajectory-related interneurons of low anxiety rats $Z = 6.56$ and $p < 0.001$). Error bars represent mean + /- SEM. F In the closed arms, a positive correlated activity is observed between anxiety-related pyramidal neurons and trajectory-related interneurons (Pearson's correlation, left, neurons from high anxiety rats, right, neurons from low anxiety rats). Pyramidal neurons population activity plotted over interneurons population activity (colours of the axes correspond to neurons as defined in B).

trajectory-related vH interneurons and anxiety-related pyramidal neurons during the exploration of the closed arms of the EPM (Fig. 5F). Collectively, these data support the idea that trajectory-related interneurons inhibit anxiety-related pyramidal neurons in an anxiety-state dependent manner during EPM navigation.

3. Discussion

While the vH is known to play a fundamental role in the genesis of anxiety responses (Fanselow and Dong, 2010; Kheirbek and Hen, 2011), its contribution to different anxiety-coping behaviours is still poorly understood. We found a reduced activation of vH pyramidal neurons in the open arms of the EPM in low anxiety rats compared to high anxiety rats. This was accompanied by a preferential trajectory-related modulation of activity in vH interneurons during EPM exploration, which selectively interacted with pyramidal neurons as a function of the anxiety state of animals.

Rats and mice placed on an EPM without pharmacological treatment,

genetic or neuronal circuit interventions are reported to spend significantly less time in the centre and open arms (2–30% of time spent in open arms) than in closed arms as high open spaces induce anxiety in rodents. In our study, using behavioural phenotyping, we generated a group of rats that predominantly explored the open arms and the centre of the EPM (up to 80% of the exploration time), after prior training to a goal-related task. Though low anxiety rats explore open arms very extensively in time and space, we provided evidence that the open spaces still trigger some anxiety in these rats (Supplementary Fig. 1B). Different variables in isolation or combined (reward-related behaviour, habituation/sensitization effects, food-deprivation, sex, handling or environmental conditions) could contribute to the 'low anxiety' phenotype (Andrade et al., 2003; Carobrez and Bertoglio, 2005). However, we did not observe habituation or sensitization after repeated exposure to the EPM (Supplementary Fig. 1 C) consistent with the innate nature of anxiety behaviour (Andrade et al., 2003; Schrader et al., 2018; Tucker and McCabe, 2017). We further presume that it is unlikely that rats confused the EPM with the food-rewarded maze as these were of different dimensions, placed at different heights, in different lighting conditions, and with different cues in the room. In addition, rodents are described as being able to discriminate well between different environments and tasks (Bartko et al., 2007; Paz-Villagran et al., 2006; Yu et al., 2018). Regarding the low anxiety phenotype, it has also been shown that food restriction per se does not alter the exploration of the open arms of the EPM in different strains of male rats (Dietze et al., 2016). Of note, EPM test sessions were always performed together with goal-related training sessions, with low anxiety rats receiving rewards, limiting the possibility of an extinction or a variable reward schedule. We propose that the low anxiety behaviour could also be driven by different coping strategies (Kent et al., 2017; Koolhaas et al., 1999; Lambert et al., 2006). It has been described that some animals react to anxiety with more active or proactive behaviours with increased movement and approach behaviour while others respond more reactively and passively, with little movement, with behaviours like freezing and avoidance (Heffer and Willoughby, 2017). We hypothesized that, in rats also experiencing goal-related behaviour, a shift towards more active and lower anxiety behaviours had occurred and that the goal-directed task contributed the most to reducing anxiety, resulting in increased open arm exploration of the EPM. Collectively, this suggests that the low-anxiety phenotype is rather influenced by goal-related training, even though the combination of different variables may have also contributed to this phenotype. Although, with our experimental approach, we were unable to address changes in physiological parameters that might reflect the extent to which low anxiety rats subjectively experienced anxiety or were rather more motivated to find potential food, distinct anxiety behaviours were associated with coherent and selective changes in vH neuronal representations.

Anxiety neurons in the vH have been shown to poorly overlap with place cells, and are consistently activated in open spaces, regardless of the animal's position (Ciocchi et al., 2015). During EPM exploration, three categories of task-responsive neurons emerged as previously reported (Adhikari et al., 2011; Ciocchi et al., 2015; Jimenez et al., 2018): 1) neurons activated in open arms reflecting anxiety, 2) neurons recruited in the centre representing approach-avoidance behaviour and 3) neurons activated in closed arms relating to safety. In accordance with previous studies and consistent with a role of the vH in mediating anxiety, we observed more pyramidal neurons representing the open spaces than the closed arms (Ciocchi et al., 2015; Jimenez et al., 2018), but we failed to detect a difference in the proportion of neurons of each category between high anxiety and low anxiety rats. Rather, the activity of pyramidal neurons activated in the open arms was lower in the first few seconds before entering or leaving the centre in low anxiety rats compared to high anxiety rats. Though we primarily investigated anxiety-related representations in the open arms of the EPM, we nevertheless identified trajectory-specific patterns in pyramidal neurons activated in the closed arms supporting the view that the closed arms of the EPM also carry task-relevant information (Supplementary Fig. 10).

In the dorsal hippocampus, the activity of pyramidal neurons has been demonstrated to undergo global and rate remapping to represent behaviourally-relevant information such as reward locations, fearful stimuli, task demands or memory content (Dupret et al., 2010; Moita et al., 2004; Sanders et al., 2019; Wood et al., 2000). In this framework, our findings of a similar fraction of task-responsive neurons suggest that the behavioural relevance of open arms as an anxiogenic experience might not be fundamentally different in low anxiety rats. Nonetheless, we noticed a rate remapping of pyramidal cell activity in low anxiety rats, which could prompt them to explore open arms much more extensively, probably owing to a different emotional state (Sanders et al., 2020).

Which mechanism might underlie differential rate remapping in pyramidal neurons activated in the open arms between low anxiety and high anxiety rats? As rats enter the open arms, monosynaptic inputs from the basolateral amygdala, a brain region instrumental for negative emotions, could drive pyramidal neurons reflecting anxiety in the vH (Grundemann et al., 2019; Liu et al., 2021; Pi et al., 2020). In turn, in low anxiety rats, memory-dependent neuromodulation of this amygdala input may lead additionally to the activation of local vH interneurons thereby reducing the activation of anxiety neurons by inhibitory interactions.

In vH interneurons, we discovered the same three neuronal categories as identified in pyramidal neurons. Of particular note, higher proportions of vH interneurons than pyramidal neurons, were specifically activated in the different compartments of the EPM, suggesting of a dense representation of vH interneurons to functional networks of the vH mediating anxiety. The proportions of vH interneurons were similar among the two group of rats, however, when inspecting their time-course of activation, we found that vH interneurons activated in the closed arms exhibited an inverse relation of their activity to pyramidal neurons activated in open arms. This opposite patterns of activation may contribute, through inhibitory or dis-inhibitory mechanisms, to the observed remapping of activity in the open arms of high anxiety and low anxiety rats.

The selective neuronal activity in the different EPM compartments could reflect distinct emotional states as previously noted, but this approach fails to fully capture meaningful features during EPM exploration. What distinguishes EPM from many other behavioural tasks is that EPM exploration relies on the animal's choice to approach or avoid a specific arm. Besides, neuronal activity patterns in the dorsal hippocampus have been shown to depend on the trajectories taken by animals, and critically, place cells have been described to be activated in only one direction during the exploration of linear mazes (Frank et al., 2000; Jercog et al., 2019; Kinsky et al., 2020; Muller et al., 1994). Taking this into account, we proposed, in our study, that a trajectory-based analysis might better reflect the different aspects of EPM exploration. We defined different trajectories based on where the animal comes from and where it goes to also considering the direction of the trajectory, either going inbound or outbound with respect to the centre of the EPM. With this analysis, we discovered a pattern of ramping activity that repeats during each trajectory which consists of an increase in activity as the animal moves inbound to approach the centre of the EPM, followed by a rapid decrease in activity during the outbound trajectory. This pattern of ramping activity was more frequently observed among vH interneurons than pyramidal neurons and was most abundant in low anxiety rats. Ramping in neuronal activity has been described in prefrontal areas where it is thought to allow for the precise timing of behaviour observed during delay and waiting periods, and ends with the initiation of motor actions (Narayanan, 2016). With respect to the hippocampus, ramping in activity has been proposed to play a role in timing as well, and has been reported in the lateral entorhinal cortex, a predominant monosynaptic input region of the vH (Banquet et al., 2021; Burwell, 2000; Tsao et al., 2018; van Groen et al., 2003). During free exploration of the EPM, timing is per se not imposed to rats to solve the task as opposed to

classical conditioning for instance. The repetitive ramping patterns reported here developed primarily in low anxiety rats that were also trained to run trajectories in a goal-related task. We thus infer that these rats may have acquired a memory with a more prominent temporal structure carried over from the goal-related task to the EPM in terms of sequencing of events (e.g., planning and execution relative to centre of the EPM). This memory could be possibly driven and maintained by serotonergic or dopaminergic neuromodulatory inputs which have been described as supporting the integration of reward and emotional memories and could potentiate specific synaptic inputs to the goal-related interneurons resulting in the observed ramping neuronal activity (Luchetti et al., 2020; Teixeira et al., 2018; Tsetsenis et al., 2021). Furthermore, suppression of the vH activity has been described to be required for sustained goal-directed behaviour (Yoshida et al., 2019) highlighting a possible role of GABAergic interneurons in inhibiting pyramidal neurons during anxiety-coping.

Along the dorso-ventral axis of the hippocampus, an increase in the number of GABAergic neurons and in their connectivity to pyramidal neurons has been reported, suggesting that this dense representation of interneurons in vH might profoundly impact information processing (Jinno and Kosaka, 2006). Numerous recent studies have shown that within the hippocampus, neuronal circuits operate in parallel by integrating selective synaptic inputs and targeting different downstream brain areas (Gergues et al., 2020; Pi et al., 2020; Sharif et al., 2021; Soltesz and Losonczy, 2018; Valero and de la Prida, 2018). CA1 interneurons have been suggested to contribute to parallel processing of information through a biased connectivity to pyramidal neurons (Lee et al., 2014), a phenomenon that could be amplified in the vH given the greater diversity of its synaptic inputs and outputs (Cenquizca and Swanson, 2007; Tao et al., 2021). Our results showed that trajectory-related vH interneurons might specifically interact with pyramidal neurons activated in open arms in an anxiety-state dependent fashion. The opposite activity of trajectory-related vH interneurons to anxiety-related vH pyramidal neurons could represent an inhibitory mechanism for emotional regulation that may affect –via selective projections from the vH to the prefrontal cortex– executive and decision-making processes. In contrast, the interaction between trajectory-related vH interneurons and anxiety-related vH pyramidal neurons was positively correlated in the closed arms of the EPM. Activity patterns in interneurons have been suggested to be correspondingly driven by local pyramidal cell assemblies (Maurer et al., 2006), though even more complex interactions have been described (Hangya et al., 2010; Wilent and Nitz, 2007). In the current study, we showed that the interaction principles between trajectory-related vH interneurons and anxiety-related vH pyramidal neurons dramatically changed from the closed to the open arms of the EPM. Genetic access for interfering with the activity of trajectory-related interneurons may represent an important approach to manipulate the transition between distinct emotional states over repeated experiences. Humans with anxiety disorders or co-morbid conditions such as schizophrenia or obsessive-compulsive disorder (Nestadt et al., 2009; Temmingh and Stein, 2015) have difficulty in regulating their emotions, planning and engaging in goal-related behaviours. Hyperactivity of the ventral/anterior hippocampus has been linked to these conditions (McHugo et al., 2019; Wolff et al., 2018) and, critically, inhibitory gating of ventral/anterior hippocampal activity has been proposed to control disease progression (Grace, 2010; Nguyen et al., 2014). Human patients with anxiety disorders are often treated with exposure therapy to fear-provoking stimuli, while experiencing these situations in a safe and controlled setting, and thus learn to better cope with their anxiety. In our model system with rats, we provide evidence that the combination of independent and interacting micro-circuits within the vH could allow for a dynamic and flexible adjustment of anxiety responses according to accumulated experience and prevailing environmental conditions.

4. Materials and methods

The data originates partially from rats ($n = 6$ low anxiety rats) that were part of a previous study (Ciocchi et al., 2015) and from additional new experiments ($n = 6$ high anxiety rats) carried out independently (Malagon-Vina et al., 2022, https://doi.org/10.1101/2022.03.22.485343). All experiments were performed in accordance with the guidelines of Medical University of Vienna and under approved licenses by the Austrian Ministry of Science. Surgeries, behavioural training and electrophysiological experiments were performed in male Long Evans rats (n = 12 rats) between 2 1/2–4 months (330–420 g) old at the time of surgery. Rats were housed individually in Plexiglas cages (42 ×27 x 30 cm) under a 12 h light/12 h dark cycle and had ad libitum access to food and water. Behavioural experiments occurred in the light phase and low anxiety rats were food deprived to reach 85% of the preoperative weight.

4.1. Surgery

Surgeries were carried out as described in (Ciocchi et al., 2015). Rats were anaesthetised with isoflurane (induction 5%, maintenance 2% in O_2) and then fixed in a stereotaxic frame and body temperature was stabilised with a heating pad. Xylocain® 2% subcutaneous and metacam® (2 mg/ml, 0.5 ml/kg) intraperitoneal were applied as local and systemic analgesics, respectively. Eye-protective cream was applied to protect the corneas. Before the skin was cut, iodine solution was used to disinfect the surgery site. Ringer's solution was administered every two hours (10 ml/kg) subcutaneously to avoid dehydration. Six stainless steel screws were anchored into the skull with two of them placed above the cerebellum to serve as grounds and references for the electrophysiological recordings. Craniotomies above the vH were performed at the following stereotactic coordinates: − 4.8 mm antero-posterior, 4.5 mm medio-lateral with respect to Bregma. A custom-made microdrive (Miba Machine Shop, IST Austria) with independently moveable tetrodes made of four gold-plated twisted tungsten microwires (12.7 μm inner diameter, California Fine Wire Company, impedances of 100–600 kΩ) was then implanted and the microdrive fixed with cement (Refobacin® Bone Cement). Dipidolor (60 mg diluted to 500 ml drinking water) was given as post-surgery analgesia. Rats recovered for at least 7 days.

4.2. Behavioural procedures

Before starting behavioural experiments, rats were handled over several days. Rats were placed on an elevated-plus-maze (EPM) for free exploration during which neural activity from the vH was recorded. The EPM consisted of two closed and two open arms measuring 9 × 50 cm and the walls in the closed arms were 40 cm high. The EPM was elevated 70 cm above the floor. Rats were placed on the EPM facing the open arm distal to the experimenter. The EPM sessions lasted 5 – 10 min and were done at 200 lux of room light intensity. The EPM sessions were of shorter duration than 10 min if rats stopped exploring the maze in order to have more comparable behavioural patterns not confounded by resting or sleeping.

The 6 high anxiety rats were only exposed to the EPM and for the 6 low anxiety rats, the EPM sessions were interleaved with training on a goal-related task on a plus maze as described in (Ciocchi et al., 2015). Briefly, rats trained on a goal-related task received a preliminary training procedure of about 1–2 weeks. The goal-related navigation task was based on consecutive learning of allocentric and egocentric rules (Ciocchi et al., 2015; Rich and Shapiro, 2009). In the allocentric task, rats learnt to find rewards at the same position at the end of one of the rewarded arms based on spatial landmarks in the experimental room. In the egocentric rule, rats learnt to find rewards by turning in the same direction from each of the start arms and therefore ending at opposing rewarded arms. Rats were first habituated to the maze and to continuously run trials for sucrose pellets (3 ×20 mg, TestDiet) on a plus maze

with two opposing starting and rewarded arms meeting at 90° angles. Sucrose pellets were delivered by sensor-activated dispensers (Campden Instruments Ltd) at the extremity of each rewarded arm. The size of the arms was 80 cm × 11 cm and the plus maze was 55 cm high and wooden-made. Both rewarded arms were baited during maze habituation but only one arm during rule learning. Training to allocentric and egocentric rules started once rats continuously performed 40–60 trials during the habituation phase. The light intensity in the experimental room during rule switching task was 1 lux to mitigate light-induced anxiety.

The task and EPM sessions were performed in the same room. The room configuration was changed by using different landmarks and configuration of the screens surrounding the mazes. The rats' position was monitored using an array of LEDs of three different colours detected at 25 frames per second by an overhead video camera (Sony). As the number of entries into the arms did not differ significantly between high and low anxietyrats (Supplementary Fig. 1 A; two-way ANOVA) but the difference in spatial exploration of open arms was large and persistent (Fig. 1B, Supplementary Fig. 1 C) we focussed our analysis of the EPM behaviour in the time domain with respect to entry and exit from the centre. We determined that, to have comparable sampling between the two rat groups, 4 s as sampling periods in the arms (after and before a centre visit using 1 s bins) could be analysed because the extent of time bins explored between the rat groups did not differ significantly over 4 s (Fig. 1C). As the time spent in the centre for each visit was variable (ranging from 0.32 s to over 80 s), we chose to analyse our data 1 s after entering and 1 s before leaving the centre.

4.3. Electrophysiological recordings and data processing

Tetrodes were progressively lowered to the vH over a period of about 3 weeks by using SWR and theta oscillations as electrophysiological hallmarks. For each recording day, tetrodes were moved to sample new units.

The extracellular electrical signals from the tetrodes were pre-amplified with a headstage (HS-132A, 2 ×32 channels, Axona Ltd). The output signals were amplified 1000X via a 64-channel amplifier and continuously digitised at 24 kHz at 16 bit resolution using a 64-channel analogue-to-digital converter computer card (Axona Ltd). The signals were down-sampled offline at 20 kHz. Spike waveforms from individual neurons were detected using the KlustaKwik automatic clustering software (http://klustakwik.sourceforge.net/). Individual single-units were selected manually by verifying the waveform shape, the modulation of waveform amplitude across tetrode channels, stability in time and the temporal autocorrelation (to assess the refractory period of a single-unit) and crosscorrelation (to assess a common refractory period across single-units) using the Klusters software (Harris et al., 2000).

4.4. Spike classification into putative pyramidal neurons and interneurons

The classification of spikes to putative pyramidal neurons and interneurons was principally based on average spike width and spike shape, and additionally on firing rate and the 1st moment of autocorrelation (Csicsvari et al., 1998; Riera et al., 2014). An adjusted spike width (adjspkw) for each spike cluster was calculated incorporating the spike width and spike shape symmetries (Sirota et al., 2008) (see also Supplementary Fig. 2):

$$adjspkw = w * \frac{\left((1 + spkScore1) + \left(1 - \frac{1}{spkScore2} + 1\right)\right)}{2}$$

w: spikewidth at 25% from baseline.

spkScore1 = peak1 to peak2 difference (=pp) of averaged spike shape normalized. if spkScore1 < 0, spkScore1 = 0.

spkScore2 = ratio of trough to peak2 time / peak1 to trough time (tp/pt) of average spike shape, if spkScore2 < 1, spkScore2 = 1.

The adjusted spike widths of the recorded neurons showed a bimodal distribution which could be clustered in Matlab using agglomerative hierarchical clustering. Neurons with an adjusted spike width below 0.26 ms and firing rates greater than 1 Hz were classified as putative interneurons. Neurons with an adjusted spike width greater than 0.26 ms and firing rates between 0.05 and 20 Hz were classified as putative pyramidal neurons. 2 outliers from these criteria (adjusted spike width greater 0.26 ms and firing rate greater 20 Hz) were further tested for their propensity to fire action potentials as bursts by calculating the 1st moment of autocorrelation. If the 1st moment of the autocorrelation fell below 10 ms, the neuron was designated as a bursty cell; if greater than 10 ms, the neuron was designated as a wide-spiking interneuron (Fuentealba et al., 2008). Neurons not meeting these criteria or displaying an unusual spike width below 0.08 ms or greater than 0.45 ms (202 neurons out 852), were excluded from our analysis to keep stringent criteria for neuronal classification.

4.5. Trajectory analysis

A rat's trajectory was defined as the exploration of a specific EPM arm (one out of the four), followed by the return to the centre of the EPM, and with a consecutive visit of any of the four EPM arms (in certain trajectories, the same arm can be visited twice after returning to the centre of the EPM). The minimal time for an arm visit was set at 300 ms. In space, a trajectory was defined from the most distally explored spatial bin of a specific EPM arm to a consecutive most distally explored spatial bin visit of any of the four EPM arms (taking into account directionality). In time, the trajectories were defined by referencing the entries and exits with respect to the centre. To compare datasets, trajectories in time were including 1 s of centre exploration before or after an EPM arm visit considered for 4 s. These analyses used time bins of 1 s. Trajectories in space were linearized and binned (5 bins of 9 ×10 cm bin size each). Four trajectory types were defined on the EPM: from an open arm to a closed arm, from a closed to an open arm, from an open to an open arm and from a closed to a closed arm. The two open arms and two closed arms were combined together.

For the comparisons of inbound to outbound trajectories, each trajectory type (as defined above) was equally represented by averaging each trajectory type to avoid an overrepresentation of specific trajectories (in case they occurred more frequently).

4.6. Tissue processing

To confirm the position of the recording sites, lesions were made at the tip of the tetrodes using a 30 μA unipolar current for 10 s (Stimulus Isolator, World Precision Instruments). Rats were then deeply anesthetised with urethane and perfused with saline followed by 20 min fixation with 4% paraformaldehyde, 15% (v/v) saturated picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer. Serial coronal sections were cut at 70 μm with a vibratome (Leica). Sections containing a lesion were Nissl-stained.

4.7. Quantification and statistical analysis

The data on spikes were further analyzed with Matlab using the Statistics and Machine Learning toolbox and custom scripts written by the authors if not otherwise stated. The behavioural analysis of the distance travelled, time spent and arm entries were analysed with a two-way ANOVA with post-hoc comparisons in Matlab. Differences in the neuronal activity in space or time bins between high anxiety and low anxiety rat were tested with shuffling statistics. To do so, the identity of rats (e.g. high anxiety vs. low anxiety) was shuffled and difference calculated 10000 times. A bin was determined as significantly different if the original difference was greater than 99.5% or lower than 0.5% of the shuffled difference (two-sided statistical test with alpha as 0.01). Chi-squared tests were used to compare if proportions of neurons were

different between groups of rats (alpha = 0.05, prop_test matlab script by Laurie 2021). The neurons were assigned to specific populations (activated in open, centre or closed compartments of the EPM) based on binned neuronal activities in time for trajectories in the closed, centre and open compartments (4 s in specific arm type and 2 ×1 s in the centre). These samples were non-parametric and therefore statistically analysed with a Mann-Whitney U test with an alpha Bonferroni corrected for multiple comparisons set to 0.0167 (three categories to be compared: closed vs open, closed vs centre, open vs centre). Significance in inbound to outbound trajectories was statistically evaluated with paired Wilcoxon signed rank tests (alpha = 0.05). For comparison of unequal groups, a Mann-Whitney U test was used as stated in the figure legends (with an alpha = 0.05 if not stated otherwise). For non-parametric test, the Z-value is also given as a standard score with an approximation of the test statistic (U or W) when comparing larger samples.

For quantifications performed on neuronal populations, z-scored neuronal activities were calculated to allow unbiased comparisons of mean firing rates. Each neuron was independently z-scored based on its activity in the whole recording session. To account and correct for a correlation between speed and neuronal activity a GLM of the z-scored neuronal activities was fitted in Matlab (glmfit) for neurons that were significantly speed correlated (Pearson's r with $p < 0.05$). The residual neuronal activity of the GLM (i.e. the neuronal activity not explainable by variations in speed as determined by the fitted GLM) was used in subsequent analysis. To illustrate data variability in graphs, standard error of the mean (SEM) was used.

5. One sentence summary

Distinct anxiety behaviours are associated with differential activity of ventral hippocampal interneurons.

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Author contributions

T.F., E.V., H. M-V., T.K., and S.C. conceived the project and designed experiments. S.C. performed the experiments and collected data. T.F. analysed data with help from E.V., H. M-V., T.K., T.N. and S.C. T.F., E.V., H. M-V., T.K., T.N. and S.C. contributed to the writing of the manuscript. S.C., T.K., T.N. acquired funding and S.C. supervised the project.

Declaration of Competing Interest

The authors declare no competing interests.

Data Availability

Any original data or additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pneurobio.2022.102368.

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