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Fronto-temporal galanin modulates impulse control

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Abstract

Rationale: The neuropeptide galanin has been implicated in a wide range of pathological conditions in which frontal and temporal structures are compromised. It works through three subtypes of G-protein-coupled receptors. One of these, the galanin receptor 1 (Gal-R1) subtype, is densely expressed in the ventral hippocampus (vHC) and ventral prefrontal cortex (vPFC), two brain structures that have similar actions on behavioral control. We hypothesize that Gal-R1 contributes to cognitive-control mechanisms that require hippocampal-prefrontal cortical circuitry.

Objective: To examine the effect of local vHC and vPFC infusions of M617, a Gal-R1 agonist, on inhibitory mechanisms of response control.

Methods: Different cohorts of rats were implanted with bilateral guide cannulae targeting the vPFC or the vHC. Following infusion of the Gal-R1 agonist, we examined the animals' behavior using a touchscreen version of the 5-choice reaction time task (5-Choice task).

Results: The Gal-R1 agonist produced opposing behaviors in the vPFC and vHC, leading to disruption of impulse control when infused in the vPFC but high impulse control when infused into the vHC. This contrast between areas was accentuated when we added variability to the timing of the stimulus, which led to long decision times and reduced accuracy in vPFC group but a general improvement in performance accuracy the vHC group.

Conclusions: These results provide the first evidence of a selective mechanism of Gal-R1 mediated modulation of impulse control in prefrontal-hippocampal circuitry.

Keywords

Impulsivity; behavioral inhibition; prefrontal cortex; hippocampus; attention; galanin; M617

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Introduction

Recent studies in rats have examined the function and connections of the ventral hippocampus (vHC), which we now believe to be at least as important as the ventral prefrontal cortex (vPFC) for certain aspects of behavioral control. For example, “prefrontal” deficits emerge following lesions to the vHC and can be pronounced even when the prefrontal cortex is intact (Mariano et al., 2009; Abela et al., 2013; Abela and Chudasama, 2013). Moreover, ‘disconnecting’ these regions following combined unilateral lesions in opposite hemispheres points to an obligatory intrahemispheric interaction between the vHC and vPFC underlying the proper control of impulsive and perseverative behavior (Chudasama et al., 2012). In addition to the effects of lesion studies and their known anatomical connections (Swanson, 1981; Groenewegen et al., 1987; Vertes 2006; Prasad and Chudasama, 2013), their shared ascending catecholamine signals may help explain why the vHC and vPFC are so closely linked. Classical neurotransmitters are the main chemical messengers, but they co-exist with neuropeptides (de Wied and de Kloet, 1988), which are increasingly recognized as modulators of cognitive pathways (Ögren et al., 2010). Of particular relevance is the neuropeptide galanin (Gal) which has been implicated in a wide range of pathological conditions in which frontal and temporal structures are compromised (Rodriguez-Puertas et al., 1997; Kovac and Walker, 2013; Juhasz et al., 2014).

Like all neuropeptides which co-localize with other neurotransmitters (for review, see Lang et al., 2015), Gal is found in high levels in noradrenaline-positive neurons of the locus coeruleus (LC), a structure known to play a prominent role in aspects of attention and arousal (Aston-Jones and Bloom, 1981; Carli et al., 1983; Cole and Robbins, 1992). In the LC, Gal is expressed in 80–90% of the neurons (Holets et al. 1988; Robinson 2004; Jacobowitz et al. 2004), and the LC constitutes the main source of Gal to the vHC and vPFC (Hökfelt et al., 1998; Xu et al., 1998). Thus, LC-derived galanin released into these structures may participate in cognitive-control mechanisms that require hippocampal-prefrontal cortical circuitry.

Most of the the reported effects of Gal on cognition are from tests of spatial navigation such as the Morris water maze with an emphasis on hippocampal mediated memory (Crawley and Wenk, 1989; Ögren and Pramanik, 1991; Ögren et al., 1996; Schött et al., 1998; Robinson and Crawley, 1994). This focus can be traced to the discovery that Gal is overexpressed in the basal forebrain of Alzheimer’s patients (Beal et al. 1990; Bowser et al. 1997; Chan-Palay 1988), and that Gal acts to inhibit cholinergic release in the hippocampus (Crawley, 1993; Robinson et al., 1996) causing mnemonic failures (Crawley, 1993; Robinson et al., 1996; McDonald et al. 1998). Like all neuropeptides, Gal is a neuromodulator that, depending on the circuit, can have either net inhibitory or net excitatory effects (Jhamandas et al., 2002). This might explain why Gal modulation of the cholinergic system does not explain all Gal-mediated learning deficits (Sabbagh et al., 2012).

Since galanin receptor 1 (Gal-R1) and galanin receptor 2 (Gal-R2) are differentially expressed within the hippocampus with Gal-R1 expressed densely in the ventral CA1 only (Weinshenker and Holmes 2016; O’Donnell et al. 1999), local infusions of Gal in the dorsal and ventral hippocampus might impact cognitive behavior in different ways (Ögren et al.,

1999; 2010). Importantly, although the vHC is recognized as the hippocampal region most associated with emotional anxiety (Bannerman et al., 1999, Moser and Moser, 1998; Kjelstrup et al., 2002), the important role of this structure in the inhibition of inappropriate actions has long been known (Jarrard and Isaacson, 1965; Nonneman et al., 1974; Rawlins et al., 1985; see also, Gray and McNaughton, 1983). How Gal-R1 in the vHC contributes to this role, and how it compares with Gal-R1 in the vPFC, remains unknown.

In this study we approach this topic by using an intra-cranial microinfusion procedure in rats performing a complex behavioral task that requires a high level of executive control. Specifically, we examined the effects of infusing a Gal-R1 agonist, M617, directly into the rat vHC on performance of the 5-choice reaction time task (5-Choice task), a task in which optimal performance requires stringent control of impulsive and compulsive responses. Our results demonstrate that Gal-R1 stimulation produces opposing behaviors in the prefrontal cortex and hippocampus, leading to disruption of impulse control when infused in the vPFC but unusually high impulse control when infused into the vHC.

Methods

Subjects.

Subjects were male Long-Evans rats (Envigo, Indianapolis, IN, USA) housed in pairs in a temperature-controlled room (23.3 °C) under diurnal conditions (12:12 h light: dark). Rats weighed between 250 and 275 g at the start of behavioral training and were maintained at 85% of their free-feeding weight throughout the experiment. All testing occurred at a regular time during the light period. All experimental procedures were approved by NIMH Institutional Animal Care and Use Committee, in accordance with the NIH guidelines for the use of animals.

Apparatus.

Six automated touchscreen operant chambers (Lafayette Instrument Company, Lafayette, IN, USA) were used, each comprising a standard operant chamber fitted with a touchscreen. Each chamber was individually housed within a sound-attenuating cabinet, ventilated by low-level noise fans, and illuminated by a 3W house light mounted on the ceiling of the sound-attenuating cabinet. The ceiling and two sidewalls of the operant chamber were made of clear Plexiglas (one of which served as the door). One side of the operant chamber was fitted with a touch-sensitive monitor (9"W × 10"H). Opposite the touchscreen, on the rear wall, was a food magazine attached to a pellet dispenser. The food magazine could be illuminated with a light emitting diode. Magazine entries were detected by a photobeam located at the entrance of the food magazine. Sucrose pellets served as food reward (Dustless Precision Pellets, Bioserve, NJ, USA).

Images of white squares were presented at one of five discrete locations on the touchscreen (Fig. 1a). A black mask, approximately 1 cm from the surface of the display, served to restrict the rat's access to the display except through response windows each measuring 2.06"L × 2.06"H. The apparatus and online data collection for each chamber were controlled by a Dell computer connected to an Animal Behavior Environmental Test (ABET) software

(Lafayette Instruments Company, Lafayette, IN, USA) interfaced with the Whisker control system for research (Cardinal and Aitken 2010).

Behavioral procedure

Pretraining.

Rats were first given one or two sessions in which they were allowed to habituate to the chamber and trained to collect pellets that were delivered every 10 seconds concomitant with illumination of the food magazine. Once rats were reliably retrieving and consuming pellets, they were trained to touch the screen. A white square (2"× 2") was presented in all five locations. The stimuli remained on the screen until the rat made a response. A nosepoke touch response to any of the five squares resulted in the disappearance of the stimuli concomitant with the delivery of a sucrose pellet and the illumination of the food magazine (~ 2 to 3 sessions). The next trial started after a 2 second interval during which the rat could not be in the food magazine. When rats were able to make ~70 touches within 35 mins, rats were presented with a single square stimulus in one of the 5 locations, and a touch in the right location was rewarded, while incorrect responses had no consequence (~ 1 session). Five seconds after making a food magazine entry, a stimulus appeared on the screen.

5-Choice Training.

Rats were trained to detect a brief visual stimulus presented pseudorandomly in one of five spatial locations. An illustration of the structure of the task is presented in Fig 1b. At the beginning of the training session, the food magazine was illuminated and the rat initiated a trial by making a food magazine entry. Following a fixed 5 s intertrial interval (ITI), a white square stimulus was presented in one of the five locations for 0.5 s. Correct responses in this location during the stimulus presentation or within a 5 s response window were rewarded with the delivery of a single pellet concurrent with the illumination of the food magazine followed by 5 s consumption time. Responses in an empty location where a stimulus was not presented (incorrect response) or a failure to respond within the response window (omission) were not rewarded and punished with a 5 s time-out during which the houselight was turned on and the entire chamber was illuminated. Responses during the 5 s interval before the onset of the target stimulus were recorded as premature responses and also punished with a 5 s time-out. Additional responses in any location following a correct response were recorded as perseverative responses. The next trial, signaled by the illumination of the food magazine started 2 s after the the consumption or the time-out period, during which the rat had to refrain from entering the receptacle.

During any one session, the white square stimulus was presented an equal number of times in each of the 5 locations in a pseudorandom order. A daily session consisted of 100 completed trials or was terminated after 35 min of testing. For the first sessions of training, both the stimulus duration and the response window were set at 10 s. These variables were altered on subsequent sessions according to the individual animal's performance until the target set of task parameters could be instituted. The target parameters were: stimulus duration, 0.5 s; response window 5 s; ITI, 5 s; time-out 5 s. The animals were considered to have reached a baseline schedule of performance when the following target parameters were

attained on 5 consecutive sessions: >80% correct responses and <20% omissions within the 35-min session time (~ 10 sessions). Once rats had acquired this training criterion, they were implanted with bilateral guide cannulae in the vPFC or the vHC. Following a two week recovery period, animals were retested on the baseline schedule of the task until they reached stable criterion performance (~ 7 sessions).

Surgery and cannulae implantation

In one group of rats, double guide cannula (26-gauge, inner diameter: 0.26 mm, outer diameter: 0.46 mm, 1.5 mm apart, projecting 7 mm from the pedestal) were implanted in the mPFC (Plastics One, Roanoke, VA, USA). Another group of rats were implanted with bilateral guide cannulae (22-gauge, inner diameter: 0.41 mm, outer diameter: 0.71 mm, projecting 9 mm from the pedestal) in the vHC (Plastics One, Roanoke, VA, USA). Animals were anaesthetized with isoflurane gas (5% induction, 2% maintenance) and placed in a stereotaxic frame fitted with atraumatic ear bars (David Kopf Instruments, Tujunga, CA, USA). The incisor bar was set at - 3.3 mm. A small quantity of ophthalmic ointment (LubriFresh, Major, Livonia, MI, USA) was gently wiped over each eye to prevent desiccation of the corneal surfaces. The scalp was retracted to expose the skull and craniotomies were made directly above the target region of the brain. The guide cannulae were carefully lowered through the craniotomies at the following coordinates taken from Paxinos and Watson (2005): mPFC (AP + 3.24, ML 0.7, DV -3.2 from dura); vHC (AP -5.6, ML 5.0, DV -7 from dura). The cannulae were affixed with dental cement and stainless sterile screws that served to hold the cannulae in place. Sterile (dummy) stylets (Plastics One, Roanoke, VA, USA) cut so they were flush with the end of the guide cannulae prevented occlusion.

Microinfusion procedure

Rats were first adapted to two mock infusions to minimize any stress associated with the procedure. Rats were gently restrained while the dummy stylets were removed and replaced with a 28 gauge double injector for vPFC or two single injectors for the vHC extending 1 mm beyond the tip of the guide cannula (Plastics One, Roanoke, VA, USA). The injectors were connected by Portex fine bore polythene tubing (0.23X.050; Plastics One, Roanoke, VA, USA) to two 5 µl precision syringes (SGE analytical science, Melbourne, Australia) mounted on an infusion pump (Harvard Apparatus, Holliston, MA, USA). Drug or vehicle were infused bilaterally in a volume of 0.5 µl over 2 min. The injectors were left in place for a further 30 s before behavioral testing.

Drug preparation and experimental design

Fig. 1c provides a schematic of the experimental design. Following cannulae implantation and postoperative recovery, animals were retested on the standard baseline schedule of the task until they reached stable criterion performance. During the baseline schedule, the ITI duration for every trial was set to 5 s. The galanin receptor 1 agonist M617 (Tocris Bioscience, Minneapolis, MN, USA), was prepared by dissolving it in 0.9% sterile saline. Animals were infused with a counterbalanced bilateral infusion of saline, 3 or 5 nmol dose

of M617, and tested on the standard baseline schedule of the task. The doses were chosen based on the literature (Kong and Yu, 2013; Scott et al. 2000). Following one week of no testing, the animals were returned to the baseline schedule for one day, and then challenged with the high 5 nmol dose and tested on a version of the task in which the stimulus was made unpredictable. In these sessions, the ITI was made variable ranging from 0.5 s to 15 s. The value of the ITI was pseudorandomly selected such that each session comprised a mixture of both short and long ITIs. To account for the long intervals, the session length was extended to last for 1 hour or terminated after 100 completed trials, whichever came first. Drug test days were preceded by a baseline training day and followed by a drug free day of no testing.

At the conclusion of behavioral testing, the rats were perfused transcardially with a working solution of PBS (1X) followed by 4% paraformaldehyde in phosphate-buffered saline. The brains were extracted and postfixed in 4% paraformaldehyde. After dehydration by immersion in 30% sucrose, the brains were cryo-sectioned at 60 to 100 μ m thickness. Every other section was mounted on glass slides and stained with cresyl violet. The sections were used to verify cannulae placement.

Performance measures

The structure of each trial in the 5-choice task and the basic measurements are depicted in a schematic in figure 1c. We measured: 1) accuracy as the number of correct responses divided by the number of correct and incorrect responses, expressed as a percentage (chance performance = 20%), 2) omissions, to assess possible failures in detection and motivational/motor deficits, were defined as the proportion of trials in which no response was made relative to the total number of completed trials, and expressed as a percentage, 3) premature responses were the number of touch responses during the ITI (i.e., *before* the onset of the stimulus) measured inhibitory mechanisms of impulse control, 4) premature response latency was the time between trial initiation and the time at which the animal made a premature response, 5) perseverative responses, defined as additional responses following a correct response were calculated as the ratio as a function of the total food retrieval latency to normalise the data. The rate of perseverative responses measured inhibitory mechanisms of compulsive behavior, 6) correct response latency and incorrect response latency were defined as the time between the onset of the stimulus and the time when the animal made a response to the correct or incorrect stimulus, respectively, and 7) magazine latency was the time between a correct response and the collection of the food reward.

Data analysis

The different performance measures were extracted using a custom-written program in R, and data were subjected to a repeated measures Analysis of Variance (ANOVA) using SPSS software (SPSS Inc., Chicago, IL). Homogeneity of variance was assessed with Mauchly's sphericity test. When this requirement was violated for a repeated measures design, the F term was tested against degrees of freedom corrected by Greenhouse–Geisser to provide a more conservative p value for each F ratio. The between-subject factor was group at two levels (vHC and vPFC). The within-subjects factors was number of sessions (3 sessions for

post-operative baseline) and dose of drug at three levels (saline, 0.3nmol, 0.5nmol). Where F ratios were significant at $p < 0.05$, post hoc comparisons were made using t-tests. The variable ITI data was first pooled irrespective of ITI and analysed with paired T-tests to compare the effects of drug and saline for each group. Subsequently, group means were compared at short ITIs (0.5 – 5 s), medium ITIs (>5 – 10 s) and long ITIs (>10 – 15 s) using independent samples T-tests.

Results

The animals were first trained to criterion performance on the baseline schedule of the 5-Choice task and then implanted with bilateral guide cannulae targeting the vPFC or vHC in separate groups of rats. The schematics in figures 2a and 2b show the positions of the cannulae tips within the mPFC and vHC for each group and a representative photomicrograph illustrating the location of the cannulae tips in each group. In the photomicrographs, the Nissl stain is strongly absorbed by the surrounding tip of the cannulae which is expressed as a dark purple/blue coloration indicating the position of the cannula tip in that region.

The cannulae tips for the vPFC group were located within the ventral prelimbic and infralimbic areas in the range of 4.68 and 2.76 mm anterior-posterior to bregma. All 15 animals in this group received correct cannulae placements and were included in the final analysis. For the vHC group, the tips were located in the CA1 and ventral subiculum and ranged from - 4.68 to - 6.36 mm anterior-posterior to bregma. Four rats from the vHC group were excluded because their cannulae were incorrectly located in the entorhinal cortex. Thus, a total of 11 rats from the vHC group were included in the analysis.

Regular timing of stimulus: Gal-R1 stimulation modulates impulse control

Following cannulae placements and post-operative recovery, both groups of rats were first re-tested on the standard baseline schedule of the 5-choice task for at least seven days, without drugs, to ensure that both groups of rats were equivalent in their performance. Prior to drug infusions, the groups did not differ significantly for the last three days on the baseline schedule for performance accuracy [$F(1, 24) = 0.468$; $p > 0.05$], premature responses [$F(1, 24) = 0.727$; $p > 0.05$], rate of perseverative responses [$F(1, 24) = 2.351$; $p > 0.05$], or omissions [$F(1, 24) = 0.679$; $p > 0.05$] or latencies [all $p > 0.05$]. The animals were then subjected to a counterbalanced sequence of vehicle and drug infusions.

The main change to behavior following infusion of Gal-R1 agonist was the rate of impulsive premature responding. Specifically, the infusion led to opposite results for vPFC and vHC and varied as a function of dose, as confirmed by a dose x group interaction [$F(2, 48) = 5.72$; $p < 0.05$]. Fig. 3a shows that Gal-R1 agonist infusion into vPFC led to a dose-dependent increase in premature responding [$F(2, 28) = 3.23$; $p = 0.05$], whereas infusion into the vHC led to a dose-dependent decrease in premature responding [$F(2, 28) = 3.54$; $p < 0.05$]. The largest effect for each group followed the high 5nmol dose [$F(1, 25) = 4.82$; $p < 0.05$].

In parallel with these changes, Gal-R1 stimulation had another behavioral effect that was shared between the two injected groups [$F(1, 23) = 0.21$; $p > 0.05$], which was to decrease

the latency at which the animals made a premature response [$F(2, 46) = 4.44$; $p < 0.05$; Fig. 3b]. Importantly, the rate of perseverative responding was unaffected by Gal-R1 administration in either group [$F(1, 24) = 2.46$; $p > 0.05$] suggesting that galanin has a very specific influence on prepotent response control mechanisms.

The Gal-R1 agonist also interfered with the animals' ability to respond efficiently to the brief visual target by increasing the omission rate in the vHC-infused rats only. Thus, as shown in Fig. 3c, when the Gal-R1 was infused locally into the vHC, these rats omitted more trials relative to the vPFC group [$F(1, 24) = 6.50$; $p < 0.05$] at both doses of the drug [3 nmol: $F(1, 25) = 5.63$; $p < 0.05$; 5 nmol: $F(1, 25) = 6.79$; $p < 0.05$] but not when infused with saline [$F(1, 25) = 2.18$; $p > 0.05$]. This increase in the omission rate did not impact accuracy in the vHC group for the low dose [$F(1, 25) = 3.67$; $p > 0.05$] or high dose [$F(1, 25) = 1.31$; $p > 0.05$; Fig. 3d], and was not caused by a general level of sedation, motor or motivational changes because all animals were in the normal range in their latency to accurately detect the target [$F(1, 24) = 0.52$; $p > 0.05$] and collect their food reward [$F(1, 24) = 3.11$; $p > 0.05$].

Variable timing of stimulus: Gal-R1 stimulation in vPFC impairs attention, decision making time and impulsive control

The opposing effects of Gal-R1 stimulation on premature responding described above prompted us to further examine the effects of stimulus unpredictability in the two test groups. We delivered the highest dose (5 nmol) into the vPFC and vHC as we varied the length of the ITI between 0.5 and 15s on each trial, thus requiring the animal to wait and control its urge to respond before stimulus presentation. Each animal received a different pseudorandom sample of ITIs for the drug and vehicle condition such that no animal received the same order or distribution of intervals in any test session. We first pooled the data across all intervals to examine the overall effect of this manipulation for each group. Then, to establish how the number of premature responses were distributed as a function of the length of the ITI, we analysed the behavior during short ITIs (0.5 – 5 s), medium ITIs (>5 – 10 s) and long ITIs (>10 – 15 s). The remaining animals for the variable ITI analyses were vPFC = 8 and vHC = 10.

Adding stimulus unpredictability further disrupted task performance in the vPFC, but not the vHC, group. The number of premature responses remained high in those animals infused with the Gal-R1 agonist in the vPFC relative to saline [$t(7) = -2.34$; $p = 0.05$], whereas the rate of impulsive responses in the vHC group was not affected by stimulus unpredictability [$t(9) = 0.31$; $p > 0.05$; Fig. 4a]. As Figs. 4b and 4c show, these heightened impulsive responses were most prominent for the vPFC group, and differed most from the vHC group, for the short and medium ITI ranges [short ITIs: $t(16) = 2.92$; $p < 0.05$; middle ITIs: $t(16) = 2.39$; $p < 0.05$]. The groups did not differ at the long ITIs [$t(16) = -1.40$; $p > 0.05$; Fig. 4d].

Consistent with their impulsive state, the vPFC group were fast to make a premature response [$t(7) = 5.03$; $p < 0.05$], a finding not observed following intra-vHC infusions [$t(9) = -0.39$; $p > 0.05$; see Fig. 4e]. Since many animals in the vHC group did not commit any premature responses at the short ITI range, there were insufficient data points to analyse the premature latency data at this time point. The fast premature latency exhibited in the vPFC

group was specifically at the middle ITI: [$t(16) = -3.15$; $p < 0.01$; Fig 4g] and long ITI: [$t(16) = -4.01$; $p < 0.01$; Fig 4h].

Other aspects of performance were also impaired in the vPFC group. These animals were impaired in their ability to accurately detect the visual target [$t(7) = 2.30$; $p = 0.05$] whereas the vHC group were more accurate [$t(9) = -2.64$; $p < 0.05$]. Fig. 5a shows that the accuracy decrement in the vPFC group occurred at the middle [$t(7) = 3.61$; $p < 0.01$] and long range ITIs [$t(7) = 2.45$; $p < 0.01$]. Stimulating Gal-R1 in the vPFC also made these animals slower to make a correct response [$t(7) = -3.82$; $p < 0.01$] but only when the ITI was in the short range [$t(7) = -3.38$; $p < 0.05$]. The vHC group, on the other hand, showed higher accuracy scores when infused with the Gal-R1 agonist at the middle ITIs only [$t(9) = -3.31$; $p < 0.01$; Fig. 5b], and fast correct response latencies at the short ITI [$t(9) = 2.55$; $p < 0.05$; Fig. 5d]. In both groups, the Gal-R1 agonist had no impact on the number of perseverative responses [vPFC: $t(7) = 0.86$; $p > 0.05$; vHC: $t(9) = 1.82$; $p > 0.05$], omissions or magazine latencies (all $p > 0.05$).

Discussion

We provide the first evidence that stimulation of Gal-R1 in the vPFC and vHC modulates impulse control mechanisms, and that it does so in opposite directions. While local stimulation of Gal-R1 in the vPFC caused rats to act more impulsively, the same manipulation in the vHC caused rats to become less impulsive. When the stimulus timing was made unpredictable, stimulation of Gal-R1 in the vPFC lengthened the decision time to make a correct response and reduced accuracy in addition to their heightened impulsive state. In contrast, timing had little impact on premature responding following Gal-R1 stimulation in vHC and instead promoted a general improvement in performance accuracy. That the rate of compulsive perseverative responding was unaffected at any point in the study points to a selective mechanism of Gal-R1 mediated modulation of impulse control in prefrontal-hippocampal circuitry.

Ventral prefrontal cortex Gal-R1 mechanism in the modulation of impulse control

The behavioral changes following vPFC Gal-R1 activation share some similarities with the effects of vPFC lesions (Chudasama et al., 2003). In both cases, the rats were unable to withhold the prepotent urge to respond. Specifically, following the infusion, these animals lost their ability to schedule their behavior that would normally prepare them for the upcoming stimulus and make ready their response. This disorganization of the preparatory response occurred regardless of whether the stimulus was highly predictable, as in the easy baseline condition, or highly unpredictable as in the difficult randomized ITI condition. In the case of the latter, the rats were unable to schedule, focus and sustain their attention for even short periods of time. The impulsive urge was also expressed in the speed at which the animal made a premature response, and in some cases was accompanied with inaccurate response selection and slow decision-making. When infused with saline, these same animals showed normal control of behavior. Thus, when Gal-R1 in the vPFC is highly stimulated with an agonist, the normal restraint and control of inappropriate actions is released leading to a high impulsive state.

The normal control of impulsive behavior in the vPFC is thought to be mediated by glutamatergic transmission because infusion of the NMDA receptor antagonist [(R)-CPP] increases premature responses when infused into the infralimbic region of the vPFC (Murphy et al., 2005). One possibility is that Gal-R1, when highly stimulated, could at least partially inhibit vPFC neuron activity by acting directly or indirectly on glutamatergic transmission. The Gal-R1 is coupled to Gi/o-type proteins which inhibits the production of cAMP (Pieribone et al. 1995; Branchek et al 2000; Ma et al. 2001; Bai et al. 2018). Acting as an inhibitory neuromodulator, the stimulation of Gal-R1 on vPFC projecting pyramidal neurons could lead to a direct decrease of activity within those neurons, resulting in disinhibition of downstream mechanisms and impulsive behavior. Consistent with this idea, galanin has an inhibitory effect on noradrenaline-induced cyclic AMP response in the cerebral cortex (Nishibori et al. 1988). Since galanin co-localizes in noradrenergic nerve terminals in the vPFC, it is possible that the impulsivity induced following local Gal-R1 agonist infusions involves a reduction in noradrenergic transmission. However, there is also evidence that galanin has an excitatory role since its infusion into the medial PFC increases local extracellular noradrenaline and cAMP levels in awake rats (Yoshitake et al. 2013). It is conceivable, therefore, that the stimulatory effect of galanin on noradrenaline and cAMP levels is indirect, mediated via disinhibition of the feedback loops projecting from the vPFC to the locus coeruleus and even more densely, to the surrounding peri-locus coeruleus areas (Luppi et al., 1995).

Ventral hippocampus Gal-1R mechanism in the modulation of impulse control

We have previously shown that similar to vPFC lesions, vHC lesions cause a long-lasting increase in premature responses (Abela et al., 2013), and the conjoint interaction between the vPFC and vHC is critical for mechanisms underlying the normal control of inappropriate, disinhibited actions (Chudasama et al., 2012). Here, we found the opposite effect. Specifically, the infusion of the Gal-R1 agonist into the vHC *decreased* the number of premature responses at baseline, and *improved* rats attention to the target when the stimulus was unpredictable. So in contrast to Gal-R1 actions within the vPFC which partially mimics a lesion, Gal-R1 in the vHC seems to enhance hippocampal neural control on the behavioral response.

It is interesting to see opposite behavioral effects caused by the same receptor depending on its site of activation. These opposing effects suggest that Gal-R1 are located on different cell types or subcellular compartments in the vPFC and vHC. Establishing the exact localization of the Gal-R1 in these two regions to gauge their cell-specific contributions is currently an area of intense investigation (Berkun et al., 2019). Another explanation could be that Gal-R1 is expressed in or affect vPFC and vHC neurons belonging to neuroanatomical pathways giving rise to opposite behavioral outcomes. Several mechanisms could potentially underly Gal-R1 effects in the vHC (Mazarati et al. 2001). The excitatory response of vPFC neurons that respond to electrical stimulation of the vHC is predominantly mediated by glutamatergic stimulation of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Jay et al. 1992). Thus, the observed effects could be mediated by pre-synaptic Gal-R1 inhibitory effects on glutamatergic transmission (Zini et al., 1993) or on noradrenergic terminals as reduction of noradrenaline release in the vHC has been suggested

to diminish impulsivity (Abela et al., 2014). Infact, at low concentrations, galanin enhances the autoinhibitory effect of noradrenaline on LC neurons through alpha-2a receptors (Xu et al., 2001) which ultimately prevents overexcitation of the LC (Aghajanian et al., 1977; Svensson et al., 1977; see also Weiss et al., 2005). This suggests that the balance of LC-noradrenaline neurons is critical for normal control of behavior. One possibility is that Gal-R1 in the vHC play a central role in regulating the activity of the LC-noradrenaline system by enhancing signal to noise ratios to enable focused attention and flexible responding (Aston-Jones et al., 1999).

Galanin also reduces the release of ACh from the vHC (Fisone et al., 1987; Consolo et al., 1991) and works to inhibit cholinergic function (see Crawley and Wenk, 1989, for a review). The role of central cholinergic function in the mediation of attention and arousal is well documented (Jones and Higgins, 1995; McGaughy et al., 2002 Dalley et al., 2004) but galanin mediated reductions of ACh release in the vHC (Laplane et al., 2004) do not impact attentional mechanisms (Wrenn et al., 2006). Instead, galanin mediated reductions in ACh release in the hippocampus, are more likely to impair learning and memory functions through a Gal-R2 mechanism (Schött et al., 2000); mice lacking Gal-R1 show little if any effect on learning and memory performance (Wrenn et al., 2004). In addition to its inhibitory role, galanin can also activate and augment ACh release in cholinergic neurons projecting to the vHC leading to potential cognitive improvements (Elvander et al., 2004). It is unclear if the upregulation of galanin functions to compensate for cholinergic loss, as in the case of Alzheimer's Disease (Crawley, 1993; Hökfelt et al., 1987; Rodriguez-Puertas et al., 1997) or makes symptoms worse by creating greater presynaptic inhibition of ACh release (Counts et al., 2008). Our data would suggest that Gal-R1 mediated ACh release in the vHC has a pro-cognitive effect by reducing impulsivity and improving attention.

Final remarks

Recent studies have emphasized a functional role of galanin, as a modulator of anxiety and depression (for reviews, see Karlsson and Holmes 2006; Hökfelt et al., 2018). Both conditions have been linked with impulsivity (Jakuszkowiak-Wojten et al. 2015; Moustafa et al. 2017), and in some cases, impulsive behaviors have been shown to increase with increasing anxiety symptoms (Bellani et al., 2012; Del Carlo et al., 2012; Yu et al., 2019). The animals in this study were not tested on measures of anxiety but their normal eating, grooming and home cage social behaviors indicate that prefrontal-hippocampal Gal-R1 stimulation does not affect emotionality *per se*, but has a specific modulatory influence on impulse control behaviors.

In addition to mood disorders, alterations in galanin expression is observed in a number of pathological conditions in which frontal and temporal structures are compromised including Alzheimer's disease, epilepsy and substance abuse (Holmes et al., 2003; Genders et al., 2018; Weinshenker and Holmes, 2016). As a marker for specific neuronal populations, our data suggest that the Gal-R1 neuropeptide system may be relevant in the development, pathology or response to neuronal damage and neurodegeneration especially within frontotemporal regions. Targeting the Gal-R1 system might be of therapeutic benefit in a wide range of fronto-temporal disorders.

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Fig. 1.

Schematic illustration of (a) touch screen with rat viewing the 5-Choice visual display with one illuminated aperture, (b) the structure of the task, and (c) the experimental design.

Correct response is indicated in green font. Red font indicates inappropriate responses. ITI, intertrial interval.

Fig. 2. Diagrammatic reconstruction of coronal sections of rat brain showing cannulae placement for each rat in (a) the vPFC group (red circles) and (b) the vHC group (blue circles) with representative photomicrographs illustrating one placement for each group.

Fig. 3.

The effects of Gal-R1 agonist, M617, on performance of the 5-Choice task under standard baseline conditions when the ITI was 5 s. Stimulating Gal-R1 in the vPFC and vHC produced (a) opposing effects on the number of premature responses, (b) decreased premature response latency for both groups with increasing dose, (c) increased rate of omissions in the vHC group, and (d) did not impact performance accuracy. * $p < 0.05$

Fig. 4.

The effects 5 nmol dose of Gal-R1 agonist, M617 on measures of impulsivity when the stimulus was made unpredictable by varying the ITI. **(a)** Intra-vPFC infusion of the Gal-R1 agonist caused an overall increase in the number of impulsive premature responses, which occurred specifically when the ITI was in the **(b)** short and **(c)** middle range but **(d)** not when the ITI was long. The high premature response rate in the vPFC group was associated with **(e)** fast premature response latencies which were most evident during the middle **(f)** and long **(g)** ITI ranges. Since many of the animals in the vHC group did not commit premature responses, there was insufficient data to analyze the premature response latency for the short ITI range. * $p < 0.05$

Fig. 5.

Effects of 5 nmol dose Gal-R1 agonist, M617 on accuracy and correct latency measures of the 5 choice task when the ITI was made variable. When infused into the vPFC, the Gal-R1 agonist **(a)** impaired accuracy when the ITI was in the middle and long ranges, and **(b)** made rats slower to make a correct response when the ITI was short. Opposing effects were observed for the vHC group who were **(c)** better in terms of their accuracy scores at the middle range ITIs, and **(d)** faster in their response latencies when the ITI was short. * $p < 0.05$