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Special Issue on Galanin

Galanin impairs performance on learning and memory tasks: Findings from galanin transgenic and GAL-R1 knockout mice

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Abstract

Galanin (GAL) impairs performance on cognitive tasks when administered centrally to rats. GAL transgenic (GAL-tg) mice overexpressing endogenous GAL show deficits on the probe trial of the Morris water maze spatial learning task, on the social transmission of food preference olfactory memory task, and on the trace cued fear conditioning emotional learning and memory task. Knockout mice deficient in the GAL-R1 receptor subtype were normal on most memory tasks, while showing a small deficit in trace cued fear conditioning, suggesting a selective role for the GAL-R1 in aversive memories, and implicating other GAL receptor subtypes in spatial learning and olfactory social memory. The growing body of rodent literature implicating excess GAL in cognitive impairment is relevant to the overexpression of GAL in the basal forebrain during the progression of Alzheimer's disease.
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Galanin (GAL) inhibits the release of acetylcholine, norepinephrine, serotonin, and glutamate in brain regions important for learning and memory (Laplante et al., 2004; Ögren et al., 1996; Zini et al., 1993). This neuropeptide is overexpressed in the basal forebrain in Alzheimer's disease (AD) (Chan-Palay, 1988; Beal et al., 1990; Bowser et al., 1997; Mufson et al., 2000), where GAL-immunoreactive fibers hyperinnervate the remaining cholinergic cells in the nucleus basalis of Meynert (Mufson et al., 1993). Gaining a better understanding of the role of GAL in the modulation of learning and memory processes may help in the development of new treatment possibilities for AD.

In rodents, central administration of GAL impairs learning and memory in tests of spatial, emotional, and working memory as shown from studies using the Morris water maze, starburst radial maze, passive avoidance, conditioned fear, spontaneous alternation, T-maze delayed alternation, and delayed non-matching to position (DNMTP) tasks. Pharmacological doses of GAL impaired acquisition (Mastropaolo et al., 1988; Sundstrom et al., 1988; Ukai et al., 1995; Schött et al., 2000; Kinney et al., 2002), working memory (Robinson and Crawley, 1993; McDonald and Crawley, 1996), and

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memory consolidation (Malin et al., 1992; Kinney et al., 2003). Pharmacological blockade of GAL receptors has been shown to reverse some of the inhibitory effects of GAL on cognition. The non-selective peptidergic GAL antagonist M40 reversed GAL-induced memory deficits on the DNMTP task (McDonald and Crawley, 1996; McDonald et al., 1997). M40 also enhanced the beneficial actions of a muscarinic agonist in rats with cholinergic immunotoxin lesions on DNMTP performance (McDonald et al., 1998). Further, M35, another GAL receptor antagonist, was reported to facilitate spatial learning in the Morris water task when given alone (Ögren et al., 1992).

GAL-overexpressing transgenic mice (GAL-tg) have been developed to model the GAL overexpression in AD (Steiner et al., 2001; Kokaia et al., 2001; Holmes et al., 2003). One DNA construct, driven by the human dopamine β-hydroxylase promoter, limits the GAL overexpression to neurons containing norepinephrine or epinephrine (Steiner et al., 2001). These GAL-tg display five times higher levels of GAL peptide in the hippocampus and nine times higher in the cerebral cortex (Wrenn et al., 2002), significantly more GAL peptide in the amygdala (He et al., 2005), and a fivefold increase in GAL mRNA in the locus coeruleus (LC) as compared to wild-type littermate controls (Steiner et al., 2001). Wild-type and GAL-tg mice, bred into a C57BL/6J background, were similar on measures of general health, motor abilities, and sensory function (Steiner et al., 2001). GAL-tg mice showed reduced LTP and less glutamate and acetylcholine release from the hippocampus as compared to the wild-type controls (Mazarati et al., 2000; Laplante et al., 2004). GAL-tg mice also displayed deficits on diverse learning and memory tasks that are known to be at least partly dependent on intact hippocampal function. The Morris water task involves learning the location of a hidden platform submerged in opaque water, based on distal extra-maze cues (e.g., geometric shapes, computer, lights, experimenter, etc.). While GAL-tg mice acquired the visible and hidden platform components, they failed to display a selective search for the platform on the probe test, when the platform was removed from the pool (Steiner et al., 2001; Wrenn et al., 2002, Fig. 1(a)). This suggests that GAL-tg mice were unable to learn the specific spatial location of the platform from distant environmental landmarks during training.

Olfactory memory was tested using the social transmission of food preference (STFP) task (Galef and Stein, 1985). In this test, mice are required to remember the scent of food smelled on the muzzle of a demonstrator mouse 24 h earlier. When offered a choice of the flavored food eaten by the demonstrator mouse (cued food), or another novel-flavored food, mice with normal olfactory memory will eat a greater proportion of the familiar cued food than of the novel food. Wild-type littermates showed a significant preference for the cued food, while GAL-tg mice did not (Wrenn et al., 2003, Fig. 1(b)).

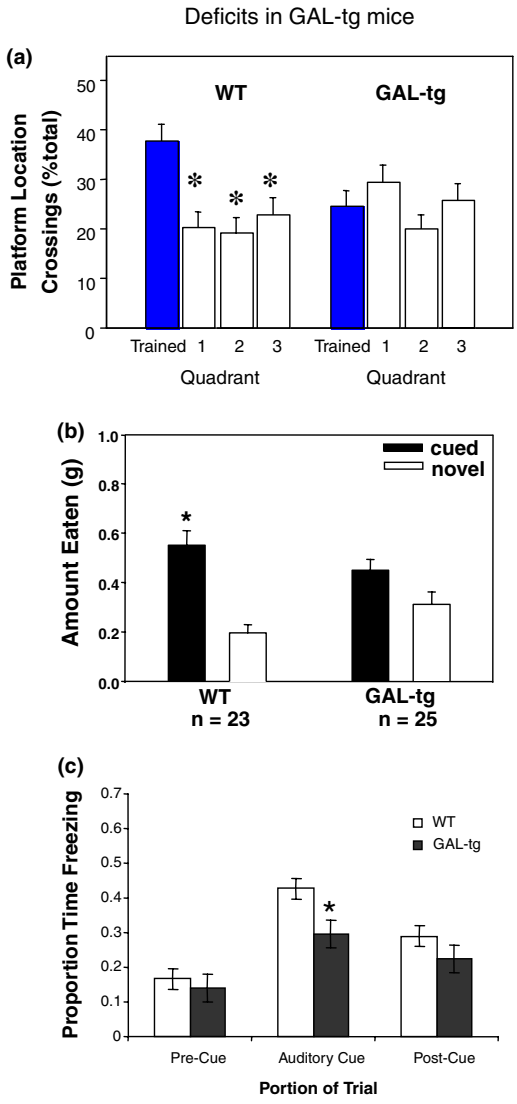

Fig. 1. Cognitive deficits in galanin overexpressing transgenic (GAL-tg) mice. (a) In the Morris water task probe trial, wild-type (WT) littermate mice showed selective quadrant search with a greater proportion of platform crossings in the trained quadrant than all other quadrants (*$p < .05$ compared to trained quadrant), while GAL-tg failed the probe trial (adapted from Steiner et al., 2001). (b) In the STFP task, WT mice showed a significant preference for the cued food (*$p < .001$) while the GAL-tg did not (adapted from Wrenn et al., 2003). (c) In trace fear conditioning, GAL-tg mice showed reduced freezing to the tone when presented in a novel context as compared to WT mice (*$p < .02$; adapted from Kinney et al., 2002).

Trace fear conditioning assays the ability of mice to learn an association between an aversive stimulus (mild footshock), the contextual cues of the testing chamber, and a tone presentation (Phillips and LeDoux, 1992). After four pairings of the tone followed 2.5 s later by the onset of footshock, freezing, defined as a lack of movement except for breathing, was measured.

Twenty-four hours after training, GAL-tg and wild-type mice were tested for their freezing response to the tone presented in a novel environment. Trace cued fear is a hippocampus-dependent response (Sutherland and McDonald, 1990; McEchron et al., 1998). Twenty-four hours following cue testing, mice were tested for freezing to the conditioning chamber, an association which is also thought to be hippocampus-dependent (Phillips and LeDoux, 1992). The genotypes did not differ in their freezing to the conditioning context, however, GAL-tg mice showed significantly less freezing than wild-type controls when presented with the tone in the novel context (Kinney et al., 2002; Wrenn et al., 2002, Fig. 1(c)). This suggests that excess GAL in the hippocampus of GAL-overexpressing mice may have interfered with the ability to form the temporal association between the discrete tone cue and subsequent footshock while still allowing the association between the ubiquitous, less discrete contextual cues and the shock.

GAL-tg mice demonstrated deficits only in the trace cued task, while the genotypes did not differ in the less demanding contextual conditioning task. This is consistent with the hypothesis that neuropeptides are co-released with neurotransmitters only when neuronal firing rates are sufficiently high (Hökfelt et al., 1987). We propose that hippocampal demand is great enough during trace cued fear and the Morris water task probe trial to cause release of excess GAL in the GAL-tg mice, leading to a greater inhibition of the cholinergic system, possibly through the inhibition of acetylcholine release (Laplante et al., 2004). As GAL can also inhibit release of glutamate (Kinney et al., 1998; Zini et al., 1993), serotonin, and norepinephrine (Kehr et al., 2001; Yoshitake et al., 2003), galaninergic modulation of these systems and the resulting effects on learning and memory warrant further investigation.

GAL has three identified receptor subtypes termed GAL-R1, GAL-R2, and GAL-R3 (Waters and Krause, 1999; reviewed in Branchek et al., 2000). All three subtypes are found in brain regions critical for learning and memory including the cortex, hippocampus, cholinergic basal forebrain, and amygdala, however, densities within these regions differ among the receptor subtypes (O'Donnell et al., 1999; Waters and Krause, 1999; Branchek et al., 2000). GAL-R1 knockout mice (Jacoby et al., 2002) were tested in the Morris water task, the STFP task, and trace fear conditioning. In the Morris water task, GAL-R1 knockout (−/−) mice showed normal acquisition curves on visible and hidden platform training as compared to wild-type (+/+) littermates and normal target quadrant search in the probe trial (Wrenn et al., 2004, Fig. 2(a)). Similarly, in the STFP task, +/+ and −/− mice showed similar preference for the cued food over the novel-flavored food (Fig. 2(b)). These results suggest that the GAL-R1 is not necessary for the formation of spatial memory or for olfactory

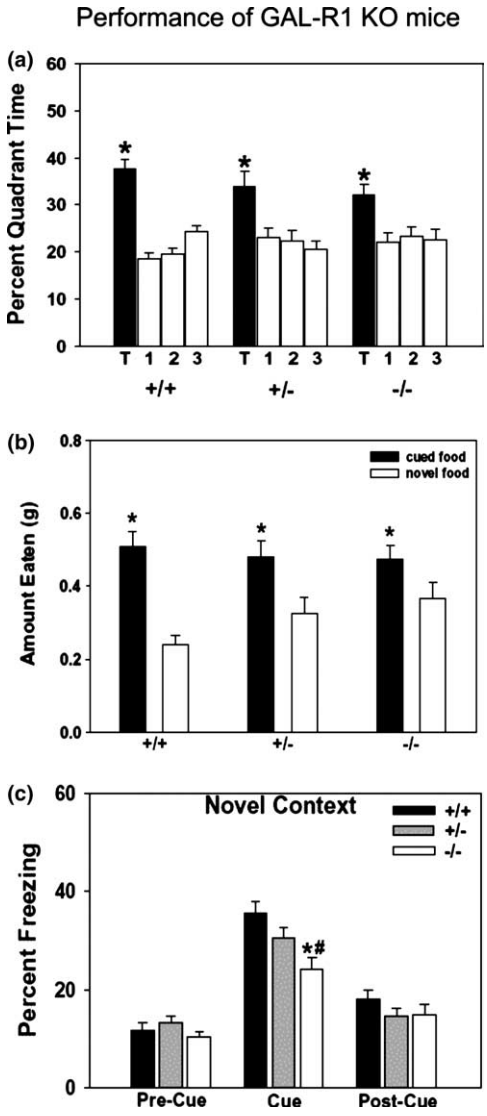

Fig. 2. Performance of galanin receptor subtype GAL-R1 knockout mice. Heterozygote (+/−) and null mutant mice (−/−) did not differ from WT mice (+/+) in (a) their preference for the trained quadrant in the Morris water task probe trial (*$p < .001$ compared to other quadrants) or (b) in their preference for the cued food in the STFP task (*$p < .0001$) (c) −/− mice showed less freezing to the tone presentation in the novel context as compared to +/+ (*$p < .001$) and +/− ($^{\#}p < .05$) mice in trace fear conditioning (adapted from Wrenn et al., 2004).

memory. In trace fear conditioning, −/− mice showed significantly less freezing than +/+ mice when presented with the auditory cue in the novel context (Fig. 2(c)), however, no differences were seen in the standard delay conditioning paradigm. These results suggest that functional GAL-R1 receptors are necessary for the formation of specific types of hippocampus-dependent memory. As the anatomical localization differs among the GAL receptor subtypes, future studies using mice deficient in GAL-R2 or GAL-R3, both at baseline and combined with GAL agonist challenges, will help to elucidate the specific roles for each of these subtypes in learning and memory processes.

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