# **Galanin Receptors in Human Basal Forebrain Differ from**<br>
The Journal of Phanet Client of the American Society for Pharmacology and Experimental Therapeutics<br> **Galanin Receptors in Human Basal Forebrain Differ from**<br>Recept **RECEPTORS IN A RECEPTOR IN THE HYPOTHAL THEOREM IN THE PRESENT CONSUMING THE CONSULTS. No. 275, No. 276, EXAPPREDICATE OF THE ADEL COLOR AND EXPERIENTAL THERMETICS**<br>
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THE 275-720-727, 1995<br> **Galanin Receptors in the Hypothalamus: Characte D. C. DEECHER,<sup>1</sup> O. O. ODUSAN and E. J. MUFSON<sup>2</sup><br>D. C. DEECHER,<sup>1</sup> O. O. ODUSAN and E. J. MUFSON<sup>2</sup><br>New Lead Discovery, Abbott Laboratories, Abbott Park, Illinois**

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# ABSTRACT

**GALACT<br>ABSTRACT**<br>Galanin, a 29-amino acid peptide, is uniquely distributed in comp<br>human basal forebrain and may play a role in cholinergic cell (M15, **ABSTRACT**<br>**Galanin, a 29-amino acid peptide, is uniquely distributed in<br>human basal forebrain and may play a role in cholinergic cell<br>dysfunction in Alzheimer's disease. We report a detailed eval**human basal forebrain and may play a role in cholinergic cell dysfunction in Alzheimer's disease. We report a detailed eval-<br>uation of galanin receptors in human basal forebrain (67  $\pm$  12 **ABSTRACT**<br>Galanin, a 29-amino acid peptide, is uniquely distributed in complements and may play a role in cholinergic cell (M15<br>dysfunction in Alzheimer's disease. We report a detailed eval- tide<br>uation of galanin recepto Galanin, a 29-amino acid peptide, is uniquely distributed in<br>human basal forebrain and may play a role in cholinergic cel<br>dysfunction in Alzheimer's disease. We report a detailed evaluation of galanin receptors in human b Galanin, a 29-animo acid pepide, is uniquely distributed in Charaman basal forebrain and may play a role in cholinergic cell (Nysfunction in Alzheimer's disease. We report a detailed eval-<br>uation of galanin receptors in h Frame pasar lotebrain and may play a role in choller given<br>dysfunction in Alzheimer's disease. We report a detailed eval-<br>uation of galanin receptors in human basal forebrain (67  $\pm$  12 high<br>years) and hypothalamus (67 aysiunction in Alzheinier's disease. We report a detailed eval-<br>
uation of galanin receptors in human basal forebrain (67  $\pm$  12 hi<br>
years) and hypothalamus (67  $\pm$  15 years) with radioligand bind-<br>
cing techniques. The diation or galariin receptors in numan basal forebrain (or  $\pm$  12<br>years) and hypothalamus (67  $\pm$  15 years) with radioligand bind-<br>ing techniques. The binding of  $[^{125}]$ galanin (porcine) (agonist) or<br> $[^{125}]$ galantide years) and hypomalamis (or  $\pm$  15 years) with radiologinal binding techniques. The binding of  $[^{125}]$ galanin (porcine) (agonist) or  $[^{125}]$ galantide [GAL (1–3)-substance P (5–11)-NH<sub>2</sub>] (putative antagonist) saturated ingled similar similar policine (agonst) of provided similar technology ( $1^{125}$ ) galantide (GAL (1-3)-substance P (5-11)-NH<sub>2</sub>) (putative reantagonist) saturated in 2 hr, and only 15% to 30% of either gradioligand was r receptors antagonist) saturated in 2 hr, and only 15% to 30% of either gior radioligand was removed in the presence of unlabeled peptide. bind  $[1^{25}][\text{Galanin} \text{ or } [1^{25}][\text{galanit}]$  or  $[1^{25}][\text{Galanin} \text{ or } [1^{25}][\text{galanit}]$  bas antagonist) saturated in 2 m, and only 13% to 30% of either gior<br>radioligand was removed in the presence of unlabeled peptide. binc<br>[<sup>125</sup>]]Galanin or [<sup>125</sup>]]galantide binding in basal forebrain re- in h<br>vealed similar radioligation was reflioved in the presence of difference peptide. Bind the particle of the presence of difference of the peptide. Bind vealed similar B<sub>max</sub> values, with  $[^{125}]$ galanin having a higher lanin affinity for

GAL is a 29-amino acid peptide isolated from the porcine<br>
Sall intestine (Tatemoto *et al.*, 1983). This peptide is w GAL is a 29-amino acid peptide isolated from the porcine St<br>small intestine (Tatemoto *et al.*, 1983). This peptide is with<br>cleaved from preprogalanin, a 123-amino acid precursor mol-GAL is a 29-amino acid peptide isolated from the porcine<br>small intestine (Tatemoto *et al.*, 1983). This peptide is<br>cleaved from preprogalanin, a 123-amino acid precursor mol-<br>ecule, to form a biologically active peptide ( GAL is a 29-amino acid peptide isolated from the porcine Stainall intestine (Tatemoto *et al.*, 1983). This peptide is with cleaved from preprogalanin, a 123-amino acid precursor molecule, to form a biologically active pep GAL is a 29-amino acid peptide isolated from the porcine Stail<br>small intestine (Tatemoto *et al.*, 1983). This peptide is with<br>cleaved from preprogalanin, a 123-amino acid precursor mol-<br>sites<br>ccule, to form a biologicall small intestine (Tatemoto *et al.*, 1983). This peptide is cleaved from preprogalanin, a 123-amino acid precursor molecule, to form a biologically active peptide (Rokaeus and Brownstein, 1986). The first 13 amino acid resi cleaved from preprogalanin, a 123-amino acid precursor molecule, to form a biologically active peptide (Rokaeus and et Brownstein, 1986). The first 13 amino acid residues of GAL S are homologous throughout the species iden ecule, to form a biologically active peptide (Rokaeus and Brownstein, 1986). The first 13 amino acid residues of GAL are homologous throughout the species identified, with residue differences occurring in the COOH-terminal Brownstein, 1986). The first 13 amino acid residues of GA are homologous throughout the species identified, with redue differences occurring in the COOH-terminal portion the sequence (Tatemoto *et al.*, 1983). The amino ac are homologous throughout the species identified, with resi-<br>due differences occurring in the COOH-terminal portion of<br>ithe sequence (Tatemoto *et al.*, 1983). The amino acid se-<br>quence of GAL has 90% homology among the sp due differences occurring in the COOH-terminal portion of its a<br>the sequence (Tatemoto *et al.*, 1983). The amino acid se-<br>incl-<br>quence of GAL has 90% homology among the species exam-<br>ined but does not share any significa the sequence (Tatemoto *et al.*, 1983). The amino acid sequence of GAL has 90% homology among the species examined but does not share any significant homology with other ader neuroactive peptides, suggesting that GAL is a quence of GAL has 90% homology among the species exam-<br>ined but does not share any significant homology with other<br>neuroactive peptides, suggesting that GAL is a member of a<br>new family of neuropeptides (Evans and Shine, 19 ined but does not share any significant homology with other<br>neuroactive peptides, suggesting that GAL is a member of a<br>new family of neuropeptides (Evans and Shine, 1991). GAL<br>protein and mRNA are widely distributed throug Received for publication March 30, 1995.<br>
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competition studies of galanin and galanin chimeric peptides<br>(M15, M35, M40 and C7) between [<sup>125</sup>l]galanin and [<sup>125</sup>l]galancompetition studies of galanin and galanin chimeric peptid<br>(M15, M35, M40 and C7) between [<sup>125</sup>l]galanin and [<sup>125</sup>l]gala<br>tide binding and in both regions. M35, M40 and C7 show competition studies of galanin and galanin chimeric peptides<br>(M15, M35, M40 and C7) between [<sup>125</sup>i]galanin and [<sup>125</sup>i]galan-<br>tide binding and in both regions. M35, M40 and C7 showed<br>high affinity for galanin receptors in competition studies of galanin and galanin chimeric peptides<br>(M15, M35, M40 and C7) between [<sup>125</sup>l]galanin and [<sup>125</sup>l]galan-<br>tide binding and in both regions. M35, M40 and C7 showed<br>high affinity for galanin receptors in competition studies of galanin and galanin chimeric peptides<br>(M15, M35, M40 and C7) between [<sup>125</sup>i]galanin and [<sup>125</sup>i]galan-<br>tide binding and in both regions. M35, M40 and C7 showed<br>high affinity for galanin receptors in competition studies or galarim and galarim chimenc peptides<br>(M15, M35, M40 and C7) between [<sup>125</sup>]galanin and [<sup>125</sup>]galan<br>tide binding and in both regions. M35, M40 and C7 showed<br>high affinity for galanin receptors in the reduced the specific binding of either radioligands investing the specific binding and in both regions. M35, M40 and C7 showed high affinity for galanin receptors in the hypothalamus with Hill coefficients close to unity, Inder binding and in Doth regions. M35, M40 and C7 showed<br>high affinity for galanin receptors in the hypothalamus with Hill<br>coefficients close to unity, whereas in the basal forebrain these<br>peptides competed differently. 5 **bigh aminy for yalarim receptors in the hypothalantis with Fill<br>coefficients close to unity, whereas in the basal forebrain these**<br>peptides competed differently. 5'-Guanylylimidodiphosphate<br>reduced the specific binding of coenicients close to unity, whereas in the basal forebrain these<br>peptides competed differently. 5'-Guanylylimidodiphosphate<br>reduced the specific binding of either radioligand in both re-<br>gions. Based on the derived data, b reduced the specific binding of either radioligand in both regions. Based on the derived data, both radioligands irreversibly bind with high affinity and act as agonists at galanin receptors in human basal forebrain and hy **teduced the specific binding of either radioligand in both r<br>gions. Based on the derived data, both radioligands irreversit<br>bind with high affinity and act as agonists at galanin recepto<br>in human basal forebrain and hypot** gions. Based on the denwi<br>bind with high affinity and<br>in human basal forebrain<br>lanin chimeric peptides (<br>tors depending on the raing subtype differences.

Staines, 1986). For the most part, the distribution of GAL<br>within the CNS correlates with the location of GAL binding Staines, 1986). For the most part, the distribution of GAL<br>within the CNS correlates with the location of GAL binding<br>sites as shown with *in vitro* receptor autoradiography (Fisone Staines, 1986). For the most part, the distribution of GAL<br>within the CNS correlates with the location of GAL binding<br>sites as shown with in vitro receptor autoradiography (Fisone<br>et al., 1987; Kohler et al., 1989; Melande Staines, 1986). For the most part, the distribution of GAL within the CNS correlates with the location of GAL binding sites as shown with *in vitro* receptor autoradiography (Fisone *et al.*, 1987; Kohler *et al.*, 1989; M Staines, 1986). For the most part, the distribution of GAL<br>within the CNS correlates with the location of GAL binding<br>sites as shown with *in vitro* receptor autoradiography (Fisone<br>*et al.*, 1987; Kohler *et al.*, 1989; M within the CNS correlates with the location of GAL binding<br>sites as shown with *in vitro* receptor autoradiography (Fisone<br>*et al.*, 1987; Kohler *et al.*, 1989; Melander *et al.*, 1988;<br>Skofitsch *et al.*, 1986). Although sites as shown with *in vitro* receptor autoradiography (Fisone *et al.*, 1987; Kohler *et al.*, 1989; Melander *et al.*, 1988; Skofitsch *et al.*, 1986). Although the exact physiological role of GAL is not clear, various Skofitsch et al., 1986). Although the exact physiological role of GAL is not clear, various studies suggest that GAL exhibition a wide range of biological responses (Hokfelt *et al.*, 1991), Skofitsch *et al.*, 1986). Although the exact physiological role of GAL is not clear, various studies suggest that GAL exhibits a wide range of biological responses (Hokfelt *et al.*, 1991), including inhibition of glucos of GAL is not clear, various studies suggest that GAL exhibits a wide range of biological responses (Hokfelt *et al.*, 1991), including inhibition of glucose-induced insulin release (Amiranoff *et al.*, 1988; Praz *et al.* its a wide range of biological responses (Hokfelt *et al.*, 1991),<br>including inhibition of glucose-induced insulin release<br>(Amiranoff *et al.*, 1988; Praz *et al.*, 1983) and inhibition of<br>adenylate cyclase, and is report (Amiranoff *et al.*, 1988; Praz *et al.*, 1983) and inhibition of adenylate cyclase, and is reported to be coupled to  $G_i$  proteins (Chen *et al.*, 1992). A recent report indicated that GAL activates at least three distin (Amiranoff *et al.*, 1988; ladenylate cyclase, and is<br>(Chen *et al.*, 1992). A rec<br>vates at least three distine<br>lison and Sharp, 1994).<br>Conflicting reports rega lenylate cyclase, and is reported to be coupled to  $G_i$  proteins<br>then *et al.*, 1992). A recent report indicated that GAL acti-<br>tes at least three distinct G proteins:  $\alpha_{i1}$ ,  $\alpha_{i2}$  and  $\alpha_{i3}$  (Gil-<br>on and Sharp, 1 (Chen *et al.*, 1992). A recent report indicated that GAL activates at least three distinct G proteins:  $\alpha_{i1}$ ,  $\alpha_{i2}$  and  $\alpha_{i3}$  (Gillison and Sharp, 1994).<br>Conflicting reports regarding the functionality of GAL an

vates at least three distinct G proteins:  $\alpha_{i1}$ ,  $\alpha_{i2}$  and  $\alpha_{i3}$  (Gillison and Sharp, 1994).<br>Conflicting reports regarding the functionality of GAL and GAL chimeric peptides in different species have impeded unde lison and Sharp, 1994).<br>Conflicting reports regarding the functionality of GAL and<br>GAL chimeric peptides in different species have impeded<br>understanding of the role of GAL pharmacology (Bartfai *et*<br> $al., 1991; Gregerson et al., 1993; Gu et$ understanding of the role of GAL pharmacology (Bartfai *et al.*, 1991; Gregerson *et al.*, 1993; Gu *et al.*, 1993; Takahashi *et al.*, 1994) and physiology (Dunning *et al.*, 1986, Gilbey *et al.*, 1989; Holst *et al.*, 1 al., 1994) and physiology (Dunning et al., 1986, Gilbey et al.,

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<sup>-</sup> Current advess: Department of Neurological Sciences and Rush Alzhel-<br>mer's Disease Center, Rush Presbyterian St. Luke's Medical Center, Chicago 1989; Holst et al., 1993; McDonald et al., 1994; Miralles et al.,<br>IL 60612. Leu-Ale-amide; C7, GAL, galanin; GLT, galantide or M15, GAL (1-13)-substance P (5-11); AD, Alzheimer's disease; K<sub>obs</sub>, slope of the line of ABBREVIATIONS: GAL, galanin; GLT, galantide or M15, GAL (1-13)-substance P (5-11) **ABBREVIATIONS: GAL, galar**<br>association; CNS, central nerve<br>Leu-Ala-amide; C7, GAL (1–13)<br>forebrain; HYP, hypothalamus.

**1995**<br>dependent, and availability of the GAL receptor cDNA It is is<br>(Habert-Ortoli *et al*., 1994) may resolve several of these is- ogy ir 1995<br>dependent, and availability of the GAL receptor cDNA<br>(Habert-Ortoli *et al.*, 1994) may resolve several of these is-<br>sues. The primary sequence of hGAL peptide was first re-1995<br>dependent, and availability of the GAL receptor cDNA<br>(Habert-Ortoli *et al.*, 1994) may resolve several of these is-<br>sues. The primary sequence of hGAL peptide was first re-<br>ported by Bersani *et al.* (1991) and short dependent, and availability of the GAL receptor cDNA It is important, therefore, to define GAL receptor pharmacol-<br>(Habert-Ortoli *et al.*, 1994) may resolve several of these is-<br>sues. The primary sequence of hGAL peptide dependent, and availability of the GAL receptor cDNA (Habert-Ortoli *et al.*, 1994) may resolve several of these issues. The primary sequence of hGAL peptide was first reported by Bersani *et al.* (1991) and shortly therea (Habert-Ortoli *et al.*, 1994) may resolve several of these is-<br>sues. The primary sequence of hGAL peptide was first re-<br>ported by Bersani *et al.* (1991) and shortly thereafter by chan<br>Evans and Shine (1991) and differs f sues. The primary sequence of hGAL peptide was first reported by Bersani *et al.* (1991) and shortly thereafter by cher Evans and Shine (1991) and differs from other known species the sequences by having an additional ser ported by Bersani *et al.* (1991) and shortly thereafter by cheroans and Shine (1991) and differs from other known species the sequences by having an additional serine residue and a dimonamidated carboxyl terminus. The add Evans and Shine (1991) and differs from other known specise<br>
sequences by having an additional serine residue and<br>
nonamidated carboxyl terminus. The additional amino ac<br>
and nonamidated carboxyl terminus may indicate that esquences by having an additional serific residue and a<br>
nonamidated carboxyl terminus may indicate that the<br>
hGAL receptor differs from receptors in other species. Inter-<br>
estingly, comparative GAL immunohistochemical (Be and nonamidated carboxyl terminus may indicate that the hGAL receptor differs from receptors in other species. Interestingly, comparative GAL immunohistochemical (Benzing *et al.*, 1993; Kordower *et al.*, 1990, 1992; Walk hGAL receptor differs from receptors in other species. Inter-<br>estingly, comparative GAL immunohistochemical (Benzing *et*<br>al., 1993; Kordower *et al.*, 1990, 1992; Walker *et al.*, 1989)<br>studies show a dramatic species di estingly, comparative GAL immunohistochemical (Benzing *et al.*, 1993; Kordower *et al.*, 1990, 1992; Walker *et al.*, 1989) studies show a dramatic species difference among monkeys, great apes and humans in the expression studies show a dramatic species difference among monkeys, great apes and humans in the expression of GAL within the subfields  $(i.e.,$  septal diagonal band complex and nucleus basalis) of the BF. For example, in monkeys, GAL studies show a dramatic species difference among monkeys,<br>great apes and humans in the expression of GAL within the<br>subfields (*i.e.*, septal diagonal band complex and nucleus<br>basalis) of the BF. For example, in monkeys, great apes and humans in the expression of GAL within the subfields (*i.e.*, septal diagonal band complex and nucleus delibrations) of the BF. For example, in monkeys, GAL colocalizes from with the BF magnocellular choline subfields (*i.e.*, septal diagonal band complex and nucleus basalis) of the BF. For example, in monkeys, GAL colocalizes with the BF magnocellular cholinergic neurons, whereas in apes and humans, this peptide is found main basalis) of the BF. For example, in monkeys, GAL colocalizes with the BF magnocellular cholinergic neurons, whereas in apes and humans, this peptide is found mainly in a separate population of small, local circuit interneu with the BF magnocellular cholinergic neurons, whereas is<br>apes and humans, this peptide is found mainly in a separate<br>population of small, local circuit interneurons (Benzing *et al.*<br>1993; Kordower *et al.*, 1990, 1992). apes and humans, this peptide is found mainly in a separate<br>population of small, local circuit interneurons (Benzing *et al.*, 1<br>1993; Kordower *et al.*, 1990, 1992). This dramatic species<br>difference in the neuronal phenot population of small, local circuit interneurons (Benzing *et al.*, panel 1993; Kordower *et al.*, 1990, 1992). This dramatic species PA difference in the neuronal phenotypic organization of gala-<br>minergic systems within th brain. **IFFORTHER EXECUTE:** The neuronal phenotypic organization of gala-<br>nergic systems within the BF suggests possible unique<br>aracteristics in GAL pharmacology within the human<br>ain.<br>Numerous biochemical, neurobehavioral and cli

minergic systems within the BF suggests possible unique characteristics in GAL pharmacology within the human and the median obtain.<br>
Numerous biochemical, neurobehavioral and clinical section<br>
pathological studies have ind characteristics in GAL pharmacology within the human<br>brain.<br>Numerous biochemical, neurobehavioral and clinical<br>pathological studies have indicated that GAL may play a key<br>role in cognitive function associated with the BF h brain. Numerous biochemical, neurobehavioral and clinic<br>pathological studies have indicated that GAL may play a ke<br>role in cognitive function associated with the BF hippocan<br>pal neuronal system. GAL has been shown to inhib Numerous biochemical, neurobehavioral and clinical<br>pathological studies have indicated that GAL may play a key<br>role in cognitive function associated with the BF hippocam-<br>pal neuronal system. GAL has been shown to inhibit pathological studies have indicated that GAL may play a key<br>role in cognitive function associated with the BF hippocam-<br>rol neuronal system. GAL has been shown to inhibit acetyl-<br>choline production within the hippocampus ( role in cognitive function associated with the BF hipp<br>pal neuronal system. GAL has been shown to inhibit is<br>choline production within the hippocampus (Fisone<br>1987) and to depress neural systems underlying w<br>memory (Crawle pal neuronal system. GAL has been shown to inhibit acetyl-<br>choline production within the hippocampus (Fisone *et al.*,<br>1987) and to depress neural systems underlying working<br>memory (Crawley, 1993) in rats. Recent immunohis choline production within the hippocampus (Fisone *et al.*, acc.<br>1987) and to depress neural systems underlying working<br>memory (Crawley, 1993) in rats. Recent immunohistochem-<br>ical (Cortes *et al.*, 1990) and *in situ* hy 1987) and to depress neural systems underlying working<br>memory (Crawley, 1993) in rats. Recent immunohistochem-<br>ical (Cortes *et al.*, 1990) and *in situ* hybridization studies<br>show a dramatic difference between humans and **with the absolute Correlation there with the memory** (Crawley, 1993) in rats. Recent immunohistochemical (Cortes *et al.*, 1990) and *in situ* hybridization studies show a dramatic difference between humans and primat ical (Cortes *et al.*, 1990) and *in situ* hybridization studies<br>show a dramatic difference between humans and primate<br>species in the neuronal phenotypic distribution of GAL<br>within the BF (Kordower *et al.*, 1990, 1992; Me show a dramatic difference between humans and primate species in the neuronal phenotypic distribution of GAL within the BF (Kordower *et al.*, 1990, 1992; Melander *et al.*, 1985; Walker *et al.*, 1991). This region is in species in the neuronal phenotypic distribution of GAL<br>within the BF (Kordower *et al.*, 1990, 1992; Melander *et al.*,<br>1985; Walker *et al.*, 1991). This region is intimately involved<br>in memory function and undergoes exte within the BF (Kordower *et al.*, 1990, 1992; Melander *et al.*, 1985; Walker *et al.*, 1991). This region is intimately involved D in memory function and undergoes extensive neuronal demonstration in AD (Mufson *et al.*, 1985; Walker *et al.*, 1991). This region is intimately involved DuP<br>in memory function and undergoes extensive neuronal de-<br>generation in AD (Mufson *et al.*, 1989; Whitehouse *et al.*, taine<br>1982). We and others have de in memory function and undergoes extensive neuronal degeneration in AD (Mufson *et al.*, 1989; Whitehouse *et al.*, 1982). We and others have demonstrated that galaninergic fibers hyperinnervate remaining cholinergic BF ne generation in AD (Mufson *et al.*, 1989; Whitehouse *et al.*, tair<br>1982). We and others have demonstrated that galaninergic sub-<br>fibers hyperinnervate remaining cholinergic BF neurons in<br>AD (Chan-Palay, 1988a; Mufson *et* 1982). We and others have demonstrated that galaninerg<br>fibers hyperinnervate remaining cholinergic BF neurons<br>AD (Chan-Palay, 1988a; Mufson *et al.*, 1993). These findin<br>have led to the suggestion that galaninergic systems fibers hyperinnervate remaining cholinergic BF neurons in AD (Chan-Palay, 1988a; Mufson *et al.*, 1993). These findings have led to the suggestion that galaninergic systems play a time in cholinergic cell dysfunction in t AD (Chan-Palay, 1988a; Mufson *et al.*, 1993). These findings have led to the suggestion that galaninergic systems play a role in cholinergic cell dysfunction in this disease (Chan-Palay, 1988b; Mufson *et al.*, 1993). Tak have led to the suggestion that galaninergic systems play a role in cholinergic cell dysfunction in this disease (Chan-Palay, 1988b; Mufson *et al.*, 1993). Taken altogether, these observations suggest that GAL may represe pharmacological strategy for AD-dependent BF dysfunction.

**It is important,** therefore, **to** define **GAL** receptor pharmacol-**Galanin Re**<br>It is important, therefore, to e<br>ogy in normal human BF.<br>The purpose of the prese

Galanin Receptors in Human Brain 721<br>is important, therefore, to define GAL receptor pharmacol-<br>y in normal human BF.<br>The purpose of the present study was to biochemically<br>aracterize GAL receptors in the human BF and to co It is important, therefore, to define GAL receptor pharmacology in normal human BF.<br>The purpose of the present study was to biochemically<br>characterize GAL receptors in the human BF and to compare<br>the pharmacology with HYP It is important, therefore, to define GAL receptor pharmacology in normal human BF.<br>
The purpose of the present study was to biochemically<br>
characterize GAL receptors in the human BF and to compare<br>
the pharmacology with H ogy in normal human BF.<br>The purpose of the present study was to biochemically<br>characterize GAL receptors in the human BF and to compare<br>the pharmacology with HYP GAL receptors of the same in-<br>dividuals with the use of an a **tive antagonist, [1251] GLT.** ML receptors in the human BF and to compare the HYP GAL receptors of the s<br>the use of an agonist,  $[^{125}I]$  pGAL, or<br> $[^{125}I]$  GLT.<br>**Materials and Methods**<br>**oreparation.** Human brains of seven men

**Materials and Methods**<br>Brain tissue preparation. Human brains of seven men (average<br>age, 56.6 years; range, 27–77 years) and five women (average age, **Materials and Methods**<br>**Brain tissue preparation.** Human brains of seven men (average age, 56.6 years; range, 27–77 years) and five women (average age, 64.2 years; range, 27–88 years) without neurological or psychiatric i **illness WETTELLE STATE 10.1 STATE 10.1 STATE 10.1 STATE 10.1 SPACE 1).** Space, 56.6 years; range, 27–77 years) and five women (average age, 64.2 years; range, 27–88 years) without neurological or psychiatric illness were Brain tissue preparation. Human brains of seven men (average age, 56.6 years; range, 27–77 years) and five women (average age, 64.2 years; range, 27–88 years) without neurological or psychiatric illness were obtained at au age, 56.6 years; range, 27–77 years) and five women (average age, 64.2 years; range, 27–88 years) without neurological or psychiatric illness were obtained at autopsy (Table 1). The average postmortem delay was 14.1 hr (ra cally, 2013 years, range, 27-88 years) without neurological or psychiatric illness were obtained at autopsy (Table 1). The average postmortem delay was 14.1 hr (range, 5–25 hr). After each brain was removed from the calvar illness were obtained at autopsy (Table 1). The average postmortem<br>floaty was 14:1 hr (range, 5–25 hr). After each brain was removed<br>from the calvaria, it was sliced coronally into 1-cm-thick slabs with a<br>calibrated Lucit delay was 14.1 hr (range, 5–25 hr). After each brain was removed<br>from the calvaria, it was sliced coronally into 1-cm-thick slabs with a<br>calibrated Lucite brain slice apparatus. The slabs were then he-<br>misected, and the ri **PA)** as described previously into 1-cm-thick slabs with a calibrated Lucite brain slice apparatus. The slabs were then he-misected, and the right hemisphere was immersion fixed in 4% paraformaldehyde solution (pH 7.4; Fis calibrated Lucite brain slice apparatus. The slabs were then hemisected, and the right hemisphere was immersion fixed in 4% paraformaldehyde solution (pH 7.4; Fisher Scientific, Pittsburgh, PA) as described previously (Muf mesoder, and the right holms (pH 7.4; Fisher Scientific, Pittsburgh, PA) as described previously (Mufson *et al.*, 1993). Samples from the left hemisphere containing the anteromedial and anterolateral subfields of the nuc PA) as described previously (Mufson *et al.*, 1993). Samples from the left hemisphere containing the anteromedial and anterolateral sub-<br>fields of the nucleus basalis  $(n = 9)$  (Mufson *et al.*, 1989) or the medial HYP  $(n =$ fields of the nucleus basalis  $(n = 9)$  (Mufson *et al.*, 1989) or the medial HYP  $(n = 5)$  were dissected, snap frozen in liquid nitrogen, and stored at  $-80^{\circ}$ C until processed for receptor binding assays. Sections from e modular 1111 W. The discussed, only in the in injure integent,<br>and stored at  $-80^{\circ}\text{C}$  until processed for receptor binding assays.<br>Sections from each brain containing the amygdala, hippocampal<br>complex and temporal cor munohistochemistry, thioflavin-S and Bielchowsky silver stains according to previously described protocols (Mufson *et al.*, 1988, 1989, 1993). Neuropathological evaluation revealed virtually no pathological examination of Alzheimer's-like degeneration (*i.e.*, neuritic plaques and neurofibrillary tangles) with Alz-50 antibody im-<br>munohistochemistry, thioflavin-S and Bielchowsky silver stains<br>according to previou pathological degeneration of the Alzheimer's type.<br>
pathological degeneration of the Alzheimer's type.<br>
pathological degeneration of the Alzheimer's type.<br> **Peptides.** GAL (rGAL, hGAL, pGAL and fGAL) Incohistochemistry, thioflavin-S and Bielchowsky silver stains cording to previously described protocols (Mufson *et al.*, 1988, 89, 1993). Neuropathological evaluation revealed virtually no thological degeneration of the manomological teachem (Torrance, CA) or Peninsula Laboratories, 1988, 1993). Neuropathological evaluation revealed virtually no pathological degeneration of the Alzheimer's type.<br>Peptides. GAL (rGAL, hGAL, pGAL and fGAL) a

1989, 1993). Neuropathological evaluation revealed virtually no<br>pathological degeneration of the Alzheimer's type.<br>**Peptides.** GAL (rGAL, hGAL, pGAL and fGAL) and GLT were<br>obtained from Bachem (Torrance, CA) or Peninsula L pathological degeneration of the Alzheimer's type.<br> **Peptides.** GAL (rGAL, hGAL, pGAL and fGAL) and GLT we<br>
obtained from Bachem (Torrance, CA) or Peninsula Laboratori<br>
(Belmont, CA). Chimeric GAL peptides M35, M40 and C7 **Peptides.** GAL (rGAL, hGAL, pGAL and fGAL) and GLT were<br>obtained from Bachem (Torrance, CA) or Peninsula Laboratories<br>(Belmont, CA). Chimeric GAL peptides M35, M40 and C7 were<br>purchased from T. Bartfai and U. Langel, (Sto botained from Bachem (Torrance, CA) or Peninsula Laboratories<br>(Belmont, CA). Chimeric GAL peptides M35, M40 and C7 were<br>purchased from T. Bartfai and U. Langel, (Stockholm University,<br>Stockholm, Sweden). [<sup>125</sup>I]pGAL and [ Methods, CA). Chimeric GAL peptides M35, M40 and C7 were<br>purchased from T. Bartfai and U. Langel, (Stockholm University<br>Stockholm, Sweden).  $[1^{25}I]GAL$  and  $[1^{25}I]GLT$  were iodinated by<br>DuPont-NEN (Boston, MA) with chlo the purchased from T. Bartfai and U. Langel, (Stockholm University, Stockholm, Sweden).  $[1^{28}I]GAL$  and  $[1^{28}I]GLT$  were iodinated by DuPont-NEN (Boston, MA) with chloramine T and lactoperoxidase methods, respectively. purchased from 1: Baluation C. Ballett, (excession entertainty, Stockholm, Sweden). [<sup>125</sup>I]pGAL and [<sup>125</sup>I]GLT were iodinated by DuPont-NEN (Boston, MA) with chloramine T and lactoperoxidase methods, respectively. The GL DuPont-NEN (Boston, MA) with chloramine T and lactoperoxidase<br>methods, respectively. The GLT used for custom labeling was ob-<br>tained from Peninsula Laboratories. Cholecystokinin (26–33) amide,<br>substance P (5–11) NH<sub>2</sub>, br the peptides (100  $\mu$ M) were in 1% (v:v) dilute acetic acid at 100  $\mu$ M, and 50- $\mu$  aliquots were stored at  $-80^{\circ}$ C until use. Stock solu-<br>  $\mu$ M, and 50- $\mu$ l aliquots were stored at  $-80^{\circ}$ C until use. Stock sol tained from Peninsula Laboratories. Cholecystokinin (26–33) amide substance P (5–11)  $NH_2$ , bradykinin (2–9) and spantide II were purchased from Bachem or Peninsula Laboratories. All stock solutions of peptides (100  $\mu$ M substance P  $(5-11)$  NH<sub>2</sub>, bradykinin  $(2-9)$  and spantide II were purchased from Bachem or Peninsula Laboratories. All stock solutions of peptides  $(100 \mu M)$  were in 1%  $(v.v)$  dilute acetic acid at 100  $\mu$ M, and 50- $\mu$ purchased from Bachem or Peninsula Laboratories. All stock solutions of peptides  $(100 \mu\text{M})$  were in  $1\%$  (v:v) dilute acetic acid at  $100 \mu\text{M}$ , and  $50-\mu$  aliquots were stored at  $-80^{\circ}\text{C}$  until use. Stock solu tions of peptides  $(100 \mu M)$  were in  $\mu$ M, and  $50$ - $\mu$ l aliquots were stores tions of peptides were thawed and mM HEPES, 1 mM MgCl<sub>2</sub>, 2 ml 0.025% NaN<sub>3</sub>, pH 7.4) for assay.<br>**Receptor preparation.** Huma  $M$ , and 50- $\mu$ l aliquots were stored at  $-80^{\circ}$ C until use. Stock solumns of peptides were thawed and serially diluted in GAL buffer (50 M HEPES, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 0.025% bacitracin and 225% NaN<sub>3</sub>, pH 7.4) placed and serially diluted in GAL buffer (50 mM HEPES, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 0.025% bacitracin and 0.025% NaN<sub>3</sub>, pH 7.4) for assay.<br>Receptor preparation. Human BF or HYP (20-25 mg/ml wet wt.) were weighed and place







**722** Deecher et al.<br>was homogenized in 35 ml of GAL buffer (50 mM HEPES, 1 mM Statist:<br>MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 0.025% bacitracin, 0.025% NaN<sub>3</sub> and 0.1% and satur<br>bovine serum albumin, pH 7.4) and centrifuged for 12 min at **722** Deecher et al.<br>was homogenized in 35 ml of GAL buffer (50 mM HEPES, 1 mM  $MgCl_2$ , 2 mM CaCl<sub>2</sub>, 0.025% bacitracin, 0.025% NaN<sub>3</sub> and 0.1% and<br>bovine serum albumin, pH 7.4) and centrifuged for 12 min at 15,000 aver<br>rp was homogenized in 35 ml of GAL buffer  $(50 \text{ mM} \text{ HEPES}, 1 \text{ mM} \text{ MgCl}_2, 2 \text{ mM } \text{CaCl}_2, 0.025\% \text{ bacitracin}, 0.025\% \text{ NaN}_3 \text{ and } 0.1\% \text{ and}$ bovine serum albumin, pH 7.4) and centrifuged for 12 min at 15,000 ava rpm (J2-21M [J-20] MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 0.025% bacitracin, 0.025% NaN<sub>3</sub> and 0.1% and saturation studies (Mcpherson, 1985) were used. Due to the bovine serum albumin, pH 7.4) and centrifuged for 12 min at 15,000 availability of age-matche bovine serum albumin, pH 7.4) and centrifuged for 12 min at 15,000 ava<br>rpm (J2-21M [J-20], Beckman Instruments, Inc., Palo Alto, CA), and vest<br>the supernatant was removed. The pellet was resuspended in 35 ml<br>of stock buff the supernatant was removed. The pellet was resuspended in 35 ml<br>of stock buffer and recentrifuged for 12 min at 15,000 rpm. The<br>supernatant was removed, and the pellet was resuspended in a<br>protume that contained  $\sim 0.47$ supernatant was removed, and the pellet was resuspended in a volume that contained  $\sim 0.47 \pm 0.09$  mg/ml protein or  $78 \pm 0.4$   $\mu$ g/200  $\mu$ l reaction volume. Protein determination was performed with the Pierce BCA prot volume that contained  $\sim 0.47 \pm 0.09$  mg/ml protein or  $78 \pm 0.4$   $\mu$ g/200  $\mu$ l reaction volume. Protein determination was performed with the Pierce BCA protein assay (Smith *et al.*, 1985). Each experiment was done wi information on individual **samples.** with the Pierce BCA protein assay (Smith *et al.*, 1985). Each experiment was done with a specific donor sample (Table 1); pooled samples were not used for this study because we wanted to collect information on individual

ples were not used for this study because we wanted to collect<br>information on individual samples.<br>**Radioligand binding protocol.** Membrane homogenate  $(100 \,\mu\text{I})$ <br>was added to each well of a 96-well microtiter plate fol **information on individual samples.**<br> **Radioligand binding protocol.** Membrane homogenate  $(100 \mu l)$  was added to each well of a 96-well microtiter plate followed by the addition of 50  $\mu l$  of binding was determined in t was added to each well of a 96-well microtiter plate followed by the addition of 50  $\mu$ l of binding buffer or unlabeled peptide. Nonspecific binding was determined in the presence of 1  $\mu$ M hGAL or GLI Reactions were in addition of 50  $\mu$ l of binding buffer or unlabeled peptide. Nonspecific binding was determined in the presence of 1  $\mu$ M hGAL or GLT. OReactions were initiated by the addition of 50  $\mu$ l of  $[^{126}I]GAL$  or n $[^{126}I]GLT$ binding was determined in the presence of 1  $\mu$ M hGAL or GLT. Or<br>Reactions were initiated by the addition of 50  $\mu$ l of  $[^{125}I]GAL$  or m<br> $[^{125}I]GLT$  in buffer, for a final reaction volume of 200  $\mu$ l. The mem-<br>branes  $[^{129}]$ GLT in buffer, for a final reaction volume of 200  $\mu$ l. The membranes were incubated (room temperature) on orbital shaker for 2 hr (25°C). Standard glass fiber filters (No. 32, Schleicher and Schuell, Keene, NH) (25°C). Standard glass fiber filters (No. 32, Schleicher and Schuell, Keene, NH) were presoaked for 1 hr in 2% polyethyleneimine before filtration to reduce nonspecific binding. The membranes were harvested on a 96-well mi Weene, NH) were presoaked for 1 hr in 2% polyethyleneimine before<br>filtration to reduce nonspecific binding. The membranes were har-<br>vested on a 96-well microtiter vacuum harvester (TomTec Mac II,<br>Orange, CT), and the unbo filtration to reduce nonspecific binding. The membranes were harvested on a 96-well microtiter vacuum harvester (TomTec Mac II, Orange, CT), and the unbound radioactivity was removed by rinsing with five cycles (3.5 ml vol **CHANNET CORRECT**<br>
Cobra gamma counter. **CODES** (Tom Tec Mac II, Corange, CT), and the unbound radioactivity was removed by rinsing<br>
with five cycles (3.5 ml vol) of 50 mM Tris-HCl wash buffer (4°C, pH<br>
7.2). Bound radioa with five cycles (3.5 ml vol) of 50 mM Tris-HCl wash buffer (4°C, pl<br>7.2). Bound radioactivity was determined with a Packard (Merider<br>CT) 10-channel Cobra gamma counter.<br>Kinetic experiments were carried out according to t

**branch was alternate was added to each well of a 96-well** microtiter were the methods of Feylund and Yamamura (1990). Briefly, an aliquot  $(100 \mu l)$  of membrane homogenate was added to each well of a 96-well microtiter o Bylund and Yamamura (1990). Briefly, an aliquot  $(100 \mu l)$  of membrane homogenate was added to each well of a 96-well microtiter plate. Total and nonspecific binding was determined for each time point. The reaction was in brane homogenate was added to each well of a 96-well microtiter GA<br>
plate. Total and nonspecific binding was determined for each time<br>
yoint. The reaction was initiated by the addition of radioligand at the<br>
set time for point. The reaction was initiated by the addition of radioligand at the time set time for association experiments. For dissociation experiments, permembranes and radioligand were preincubated for 2 hr, and at given ratime experiments and radioligand were preincubated for 2 hr, and at given<br>me points, 1  $\mu$ M GAL,  $\mu$ M GLT or 100  $\mu$ M GppNHp (Sigma Chem-<br>l Co., St. Louis, MO) was added. Membranes were harvested as<br>scribed above.<br>For membr time points, 1  $\mu$ M GAL,  $\mu$ M GLT or 100  $\mu$ M GppNHp (Sigma Cheical Co., St. Louis, MO) was added. Membranes were harvested described above.<br>For membrane wash experiments, centrifuge tubes (50 ml) containing 6 ml of hu

ical Co., St. Louis, MO) was added. Membranes were harvested as<br>described above.<br>For membrane wash experiments, centrifuge tubes (50 ml) con-<br>taining 6 ml of human BF (25 mg/ml wet wt.) membrane homoge-<br>nates were incubat described above.<br>
For membrane wash experiments, centrifuge tubes (50 ml) con<br>
taining 6 ml of human BF (25 mg/ml wet wt.) membrane homoge<br>
nates were incubated with hGAL (IC<sub>50</sub> × 100 = 1 nM) to saturation<br>
2 hr at room For membrane wash experiments, centrifuge tubes (50 ml) containing 6 ml of human BF (25 mg/ml wet wt.) membrane homogenates were incubated with hGAL (IC<sub>50</sub>  $\times$  100 = 1 nM) to saturation 2 hr at room temperature. After i taining 6 ml of human BF (25 mg/ml wet wt.) membrane homogenates were incubated with hGAL ( $IC_{50} \times 100 = 1$  nM) to saturation 2 hr at room temperature. After incubation, each tube was vortexed;<br>a 10- $\mu$ l sample was taken nates were incubated with hGAL  $(IC_{50} \times 100 = 1 \text{ nM})$  to saturation 2 hr at room temperature. After incubation, each tube was vortexed; a 10- $\mu$ l sample was taken for protein determination; and triplicate  $100$ - $\mu$ l al **a** 10- $\mu$ l sample was taken for protein determination; and triplicate 100- $\mu$ l aliquots were taken for each set of total and nonspecific samples. The volumes of the centrifuge tubes were restored to 6 m with membrane b 100- $\mu$ l aliquots were taken for each set of total and nonspecific samples. The volumes of the centrifuge tubes were restored to 6 ml with membrane buffer and centrifuged at 14,000 rpm for 10 min; the supernatant was rem samples. The volumes of the centrifuge tubes were restored to 6 ml<br>with membrane buffer and centrifuged at 14,000 rpm for 10 min; the<br>supernatant was removed, and the membrane was resuspended in<br>4.8 ml buffer. This proced supernatant was removed, and the membrane was resuspended in 4.8 ml buffer. This procedure was repeated, with  $100-\mu$ l aliquots removed from the centrifuge tubes and the volume restored to 6 ml. This wash procedure was re For acid treatment experiments, BF homogenates were incubated of a miss wash procedure was repeated 3 times. After the last wash ocedure, the final group of 100- $\mu$ l aliquots were taken, and the post of acid treatment exp For 2 hr with either radioligand. The addition of 1 ml acid solution of 2 hr with either radioligand. The addition of 1 ml acid solution (0.2 M acetic acid plus 0.2 M NaCl; 4<sup>°</sup>C) was added to the membranes

procedure, the final group of  $100-\mu l$  aliquots were taken, and the binding assay was performed as indicated.<br>For acid treatment experiments, BF homogenates were incubated for 2 hr with either radioligand. The addition of binding assay was performed as indicated.<br>
For acid treatment experiments, BF homogenates were incubated<br>
for 2 hr with either radioligand. The addition of 1 ml acid solution<br>
(0.2 M acetic acid plus 0.2 M NaCl; 4°C) was a For acid treatment experiments, BF homogenates were incubated<br>for 2 hr with either radioligand. The addition of 1 ml acid solution<br> $(0.2 \text{ M } \text{aetic acid plus } 0.2 \text{ M } \text{NaCl}; 4^{\circ}\text{C})$  was added to the membranes<br>and incubated for for 2 hr with either radioligand. The addition of 1 ml acid solution  $(0.2 \text{ M acetic acid plus } 0.2 \text{ M NaCl}; 4^{\circ}\text{C})$  was added to the membranes and incubated for 15 min  $(4^{\circ}\text{C})$ . Control samples were run in parallel and received <sup>1</sup> **2**  *M* acetic acid plus 0.2 *M* NaCl; 4°C) was added to the membranes and incubated for 15 min (4°C). Control samples were run in parallel and received 1 ml binding buffer (4°C). The reaction was centrifuged for 5 mi non-the supernation was removed, and the wash<br>protocol outlined above was followed to remove all unbound radioli-<br>gand.<br>For experiments in which the effects on radioligand binding of the<br>nonhydrolyzable GTP analogue GppNHp

protocol outlined above was followed to remove all unbound radioli-<br>gand.<br>For experiments in which the effects on radioligand binding of the<br>monhydrolyzable GTP analogue GppNHp (Sigma Chemical Co.) were<br>measured, a stock s gand.<br>
For experiments in which the effects on radioligand binding of the<br>
nonhydrolyzable GTP analogue GppNHp (Sigma Chemical Co.) were<br>
measured, a stock solution (10 mM) of GppNHp was made in binding<br>
buffer before ass For experiments in which the effects on radioligand binding of the<br>
nonhydrolyzable GTP analogue GppNHp (Sigma Chemical Co.) were<br>
ine of<br>
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buffer before as buffer.

Statistical analysia. **EBDA** analysis programs for competition Vol. 275<br>Statistical analysis. EBDA analysis programs for competition<br>and saturation studies (Mcpherson, 1985) were used. Due to the<br>availability of age-matched human tissue from each brain area in-Vol. 275<br> **Statistical analysis.** EBDA analysis programs for competition<br>
and saturation studies (Mcpherson, 1985) were used. Due to the<br>
availability of age-matched human tissue from each brain area in-<br>
vestigated, all e **Statistical analysis.** EBDA analysis programs for competition and saturation studies (Mcpherson, 1985) were used. Due to the availability of age-matched human tissue from each brain area investigated, all experiments were and saturation studies (Mcpherson, 1985) were used. Due to the availability of age-matched human tissue from each brain area investigated, all experiments were run 2 or 3 times with different samples of BF or HYP. Values e and countries the permanent studies (seepherson, 1999) were calculated and availability of age-matched human tissue from each brain area investigated, all experiments were run 2 or 3 times with different samples of BF or H availability of age-matched fiding issue from each brain area investigated, all experiments were run 2 or 3 times with different samples of BF or HYP. Values expressed are the mean  $\pm$  S.E.M. of those experiments. Statis those experiments. Statistical analysis was done on data from competition studies using the SAS statistical package (Cary, NC). A nonparametric three-factor factorial followed by the nonparametric least-significant differe of **P <.05 was considered significant.**

# **Results**

The binding of  $[{}^{125}I]GAL$  or  $[{}^{125}I]GLT$  to  $GAL$  receptors was irreversible. Kinetic experiments with  $[{}^{125}I]GAL$ **Results**<br>**Results**<br>**The binding of**  $[{}^{125}I]GAL$  **or**  $[{}^{125}I]GLT$  **to**  $GAL$  **recep-<br>tors was irreversible. Kinetic experiments with**  $[{}^{125}I]GAL$ **<br>or**  $[{}^{125}I]GLT$  **(fig. 1, top) showed**  $K_{obs}$  **rates of 0.026 and 0.042 hesults**<br>**or ['25I]GAL or**  $[$ **'25I]GLT to GAL receptors was irreversible.** Kinetic experiments with  $[$ <sup>125</sup>I]GAL or  $[$ <sup>125</sup>I]GLT (fig. 1, top) showed  $K_{\text{obs}}$  rates of 0.026 and 0.042 min<sup>-1</sup>, respectively, with BF The binding of  $[1^{26}]$ GAL or  $[1^{26}]$ GLT to GAL receptors was irreversible. Kinetic experiments with  $[1^{25}]$ GAL or  $[1^{25}]$ GLT (fig. 1, top) showed  $K_{obs}$  rates of 0.026 and 0.042 min<sup>-1</sup>, respectively, with BF homogena **tors was irreversible.** Kinetic experiments with  $[^{125}I]GAL$  or  $[^{125}I]GLT$  (fig. 1, top) showed  $K_{obs}$  rates of 0.026 and 0.042 min<sup>-1</sup>, respectively, with BF homogenates. For dissociation experiments, GAL or GLT (1 or  $[{}^{125}]$ GLT (fig. 1, top) showed  $K_{\text{obs}}$  rates of 0.026 and 0.042<br>min<sup>-1</sup>, respectively, with BF homogenates. For dissociation<br>experiments, GAL or GLT (1 $\mu$ M) or GppNHp (100 $\mu$ M) was<br>added at 2 hr, and ~90% of eit **experiments, GAL 60 GLT** (1 μm) of GppN1p (100 μm) was added at 2 hr, and ~90% of either radioligand remained bound after 1 hr. Kinetic experiments with  $[^{125}][\text{pGAL}$  or  $[^{125}][\text{GLT}$  (fig. 1, bottom) showed  $K_{\text{obs$ bound after 1 hr. Kinetic experiments with  $[^{125}I]pGAL$  or  $[^{125}I]GLT$  (fig. 1, bottom) showed  $K_{obs}$  rates of 0.017 and 0.018 min<sup>-1</sup>, respectively, with HYP homogenates. The same method as was used with BF was used wit  $[1^{25}]$ GLT (fig. 1, bottom) showed  $K_{obs}$  rates of 0.017 and 0.018 min<sup>-1</sup>, respectively, with HYP homogenates. The same method as was used with BF was used with HYP for dissociation experiments, and approximately 75% to  $0.018 \text{ min}^{-1}$ , respectively, with HYP homogenates. The same method as was used with BF was used with HYP for dissociation experiments, and approximately 75% to 80% of either radioligand remained bound after 1 hr. Dissoc method as was used with BF was used with HYP for dissociation experiments, and approximately 75% to 80% of eitheradioligand remained bound after 1 hr. Dissociation studie were continued to 4.5 hr with both radioligands in ciation experiments, and approximately 75% to 80% of eith radioligand remained bound after 1 hr. Dissociation studies were continued to 4.5 hr with both radioligands in borgions, and no change was noted (data not represen radioligand remained bound after 1 hr. Dissociation studies<br>were continued to 4.5 hr with both radioligands in both<br>regions, and no change was noted (data not represented).<br>Additional approaches were attempted to dissocia were continued to 4.5 hr with both radioligands in both<br>regions, and no change was noted (data not represented).<br>Additional approaches were attempted to dissociate  $[^{125}I]p-GAL$  or  $[^{125}I]GLT$  from the GAL receptor. BF m regions, and no change was noted (data not represented).<br>Additional approaches were attempted to dissociate  $[^{125}I]p-$ <br>GAL or  $[^{125}I]GLT$  from the GAL receptor. BF membranes<br>were incubated with  $[^{125}I]pGAL$  for 2 hr a Additional approaches were attempted to dissociate  $[^{126}]$  p-<br>GAL or  $[^{126}]$  GLT from the GAL receptor. BF membranes<br>were incubated with  $[^{126}]$  pGAL for 2 hr and then washed 4<br>times with binding buffer. A standard bin GAL or  $[^{125}]$ GLT from the GAL receptor. BF membranes were incubated with  $[^{125}]$ pGAL for 2 hr and then washed 4 times with binding buffer. A standard binding assay was performed on these membranes, and the bound to unbo were incubated with  $[1^{25}I]pGAL$  for 2 hr and then washed 4 times with binding buffer. A standard binding assay was performed on these membranes, and the bound to unbound radioligand ratios remained the same in comparison posure **of acetic acid to BF membranes preincubated with**



and received 1 ml binding buffer (4°C). The reaction was centrifuged<br>for 5 min at 12,000 rpm, the supernatant was removed, and the wash<br>protocol outlined above was followed to remove all unbound radioli-<br>gand.<br>For experim Fig. 1. Kinetics of  $[^{125}]$ pGAL and  $[^{125}]$ pGLT binding in human BF and<br>HYP. Top, 50 pM  $[^{125}]$ pGAL and  $[^{125}]$ jGLT association binding in the BF.<br>Insets, Pseudo-first-order association plots  $[^{125}]$ pGAL –  $K_{obs}$  = 0 <sup>0</sup> 60 120 180 0 60 120 180<br>
TIME (min)<br> **Fig. 1.** Kinetics of  $[^{125}]$ pGAL and  $[^{125}]$ gLT binding in human BF and<br>
HYP. Top, 50 pM  $[^{125}]$ pGAL or  $[^{125}]$ gLT association binding in the BF.<br>
Insets, Pseudo-first-order a **Fig. 1.** Kinetics of  $[1^{25}$ l]pGAL and  $[1^{25}$ l]GLT binding in human BF and<br>HYP. Top, 50 pM  $[1^{25}$ l]pGAL or  $[1^{25}$ l]GLT association binding in the BF.<br>Insets, Pseudo-first-order association plots  $[1^{25}$ l]pGAL  $- K$ **Fig. 1.** Kinetics of  $[^{125}]$  pGAL and  $[^{125}]$  pGLT binding in human BF and HYP. Top, 50 pM  $[^{125}]$  pGAL or  $[^{125}]$  jGLT association binding in the BF.<br>Insets, Pseudo-first-order association plots  $[^{125}]$  pGAL  $K$ **Fig. 1.** Kinetics of  $[^{125}]$ JpGAL and  $[^{125}]$ JGLT binding in human BF and HYP. Top, 50 pM  $[^{125}]$ JpGAL or  $[^{125}]$ JGLT association binding in the BF.<br>Insets, Pseudo-first-order association plots  $[^{125}]$ JpGAL -  $K_{obs} =$ HYP. Top, 50 pM [<sup>125</sup>l]pGAL or [<sup>125</sup>l]GLT association binding in the BF.<br>Insets, Pseudo-first-order association plots ([<sup>125</sup>l]pGAL –  $K_{obs} = 0.026$ <br>min<sup>-1</sup> and line correlation of .96; [<sup>125</sup>l]GLT –  $K_{obs} = 0.042$  min<sup>-1</sup> Insers, Pseudo-first-order association plots ( $1^{25}$ ljGLT -  $K_{obs} = 0.042$  min<sup>-1</sup> and line correlation of .96). Bottom, 40 pM ( $1^{25}$ ljDGAL or 50 pM ( $1^{25}$ l)GLT dissociation binding in the HYP. Insets, Pseudo-first-or line correlation of .95). Bottom, 40 pM  $[^{125}]$ pGAL or 50 pM  $[^{125}]$ gGLT association binding in the HYP. Insets, Pseudo-first-order association plots  $[^{125}]$ pGAL -  $K_{obs} = 0.017$  min<sup>-1</sup> and line correlation of .93;  $[^{$ association binding in the HYP. Insets, Pseudo-first-order<br>plots ( $[^{125}]$ )pGAL –  $K_{obs} = 0.017$  min<sup>-1</sup> and line correlation<br> $[^{126}]$ GLT –  $K_{obs} = 0.018$  min<sup>-1</sup> and line correlation of .9-<br>mean values of triplicate determ

<sup>1995</sup><br>either radioligand was done to determine whether the bin<br>ing of the radioligands changed the conformation of the GA ing times radioligand was done to determine whether the bind-<br>ing of the radioligands changed the conformation of the GAL<br>receptor. The addition of acetic acid to BF membranes abolreceptor. The additional was done to determine whether the binding of the radioligands changed the conformation of the GAL receptor. The addition of acetic acid to BF membranes abolished the specific binding of either radi either radioligand was done to determine whether the bind-<br>ing of the radioligands changed the conformation of the GAL<br>receptor. The addition of acetic acid to BF membranes abol-<br>ished the specific binding of either radio ing of the radioligands changed the conformation of the GAL<br>receptor. The addition of acetic acid to BF membranes abol-<br>ished the specific binding of either radioligand. To determine<br>whether the acetic acid treatment destr receptor. The addition of acetic acid to BF membranes abolished the specific binding of either radioligand. To determine whether the acetic acid treatment destroyed the receptor, the BF membranes were washed and used agai the control values (data not illustrated).<br>
BF membranes were washed and used again in a binding<br>
assay with  $[^{125}I]pGAL$ , and the specific binding was restored<br>
to control values (data not illustrated).<br>  $[^{125}I]pGAL$  an

**RF** membranes were washed and used again in a binding assay with  $[^{125}]$  pGAL, and the specific binding was restored to control values (data not illustrated).<br>  $[^{125}]$ **pGAL and**  $[^{125}]$ **GLT appeared to label the same <b>r** to control values (data not illustrated).<br>  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  appeared to label the same<br>
receptor population in basal forebrain. Due to the irre-<br>
versible binding of either radioligand,  $K_D$  and  $B_{max}$  value **EXALUATE: EXALUATE: EXALUATE: EXALUATE: EXALUATE: EXALUATE: EXALUATE: EXALUATE: EXALUATE: THE VERGINITY OF THE VERGINITY OF THE ANALYZING THE ANALYZING THE AND STATE than steady state measurements. Rose receptor population in basal forebrain.** Due to the irre-<br>versible binding of either radioligand,  $K_D$  and  $B_{max}$  values<br>are relative values based on assumed saturation (2 hr) rather<br>than steady state measurements. Rosen versible binding of either radioligand,  $K_D$  and  $B_{max}$  values  $\frac{8}{2}$  so<br>are relative values based on assumed saturation (2 hr) rather<br>than steady state measurements. Rosenthal analysis with<br> $[^{125}]pGAL$  or  $[^{125}]GLT$  i are relative values based on assumed saturation (2 hr) rather<br>than steady state measurements. Rosenthal analysis with<br> $[^{125}]$ pGAL or  $[^{125}]$ GLT in BF homogenates of aged-matched<br>control subjects (table 1) (67  $\pm$  12 ye [<sup>126</sup>I]pGAL or [<sup>126</sup>I]GLT in BF homogenates of aged-matched control subjects (table 1) (67  $\pm$  12 years old) revealed  $K_D$  values of 89  $\pm$  23 and 193  $\pm$  39 pM and  $B_{max}$  values of 35  $\pm$  8.3 and 31  $\pm$  0.7 fmol/m values of 89  $\pm$  23 and 193  $\pm$  39 pM and B<sub>max</sub> values of 35  $\pm$  8.3 and 31  $\pm$  0.7 fmol/mg protein, respectively (fig. 2, top).<br>Maximal binding densities for GAL receptors were similar for both radioligands, and  $[^{$ 8.3 and 31  $\pm$  0.7 fmol/mg protein, respectively (fig. 2, top).<br> **Maximal binding densities for GAL receptors were similar**<br>
for both radioligands, and  $[^{126}I]pGAL$  showed the highest<br>
affinity for the receptor populati for both radioligands, and  $[^{125}]$ pGAL showed the highest affinity for the receptor population labeled. Crude homogenates from two younger  $(28 \pm 1.4)$  years old) BF samples (table 1, BF 7 and 8) were also evaluated with analysis and revealed similar  $K_D$  and  $B_{max}$  values (data not represented). nates from two younger ( $28 \pm 1.4$  years old) Br samples<br>
(table 1, BF 7 and 8) were also evaluated with Rosenthal of six determinations from three experiments (table 1, BF 3, 5 and 9;<br>
analysis and revealed similar  $K_D$ 

(table 1, BF 7 and 8) were also evaluated with Rosenth<br>analysis and revealed similar  $K_D$  and  $B_{max}$  values (data n<br>represented).<br> $[^{125}]$ **pGAL labeled more receptors in the HYP tha**<br> $[^{125}]$ **GLT.** In comparison, Rosentha analysis and revealed similar  $K_D$  and  $B_{max}$  values (data not<br>represented).<br>[<sup>125</sup>I]pGAL labeled more receptors in the HYP than<br>[<sup>125</sup>I]GLT. In comparison, Rosenthal analysis with [<sup>125</sup>I]p-<br>GAL or [<sup>125</sup>I]GLT in human H **represented).**<br> **[<sup>125</sup>I]pGAL labeled more receptors in the HYP than**<br> **[<sup>125</sup>I]GLT.** In comparison, Rosenthal analysis with  $[^{125}$ ]p-<br> **GAL** or  $[^{125}$ I]GLT in human HYP of aged-matched control<br>
subjects (67 ± 15 yea <sup>[125</sup>**I]pGAL labeled more receptors in the HYP than**<br>
<sup>[125</sup>**I]GLT.** In comparison, Rosenthal analysis with  $[^{125}]$ p-<br>
GAL or  $[^{125}]$ GLT in human HYP of aged-matched control<br>
subjects (67 ± 15 years old) revealed  $K_D$  [<sup>125</sup>I]GLT. In comparison, Rosenthal analysis with [<sup>125</sup>I]p-<br>GAL or [<sup>125</sup>I]GLT in human HYP of aged-matched control<br>subjects (67  $\pm$  15 years old) revealed  $K_D$  values of 264  $\pm$  7 to or<br>and 197  $\pm$  11 pM and B<sub>max</sub> GAL or  $[^{125}][GLT]$  in human HYP of aged-matched control man tiss<br>subjects (67  $\pm$  15 years old) revealed  $K_D$  values of  $264 \pm 7$  to  $45$  pM<br>and  $197 \pm 11$  pM and  $B_{max}$  values of  $125 \pm 9$  and  $72 \pm 10$   $70 \pm 3\%$ ,<br>fmo subjects (67  $\pm$  15 years old) revealed  $K_D$  values and 197  $\pm$  11 pM and  $B_{max}$  values of 125  $\pm$  9 fmol/mg protein, respectively (fig. 2, bottom). [<sup>1</sup> a lower affinity value for the GAL receptor an more receptors in d 197  $\pm$  11 pM and B<sub>max</sub> values of 125  $\pm$  9 and 72  $\pm$  10<br>
nol/mg protein, respectively (fig. 2, bottom). [<sup>125</sup>I]pGAL had<br>
lower affinity value for the GAL receptor and labeled 42%<br>
ore receptors in this region tha

fmol/mg protein, respectively (fig. 2, bottom). [<sup>125</sup>I]pGAL had or GAL chimeric peptides. The specific binding of 40 to 45 pM<br>a lower affinity value for the GAL receptor and labeled 42% [<sup>125</sup>I]pGAL or [<sup>125</sup>I]GLT in the a lower affinity value for the GAL receptor and labeled  $42\%$ <br>
more receptors in this region than  $[^{125}][GLT]$ .<br> **GppNHp reduced the binding of**  $[^{125}][\text{pGAL or}$ <br>  $[^{125}][GLT \text{ to GAL receptors.}$  The addition of GppNHp (100 r<br>  $\mu$ M more receptors in this region than  $[^{125}][GLT]$ . 10<br> **GppNHp reduced the binding of**  $[^{125}][\text{pGAL}$  **or**<br>  $[^{125}][\text{GLT}]}$  to GAL receptors. The addition of GppNHp (100 ro<br>  $\mu$ M) reduced the specific binding of 40 to 45 **GppNHp reduced the binding of**  $[^{125}I]pGAL$  **or**  $[^{125}I]GLT$  to GAL receptors. The addition of GppNHp (100 r $\mu$ M) reduced the specific binding of 40 to 45 pM  $[^{125}I]pGAL$  or iii)  $[^{125}I]GLT$  in BF by 87% and 100%, res HYP, the specific binding of  $[^{125}I]pGAL$  or  $[^{125}I]GLT$  was reduced by 74% and 100%, respectively.



**BF GAL** BF GLT HYP GAL HYP GLT<br> **Fig. 3.** Effect of 100 μM GppNHp on [<sup>125</sup>l]pGAL or [<sup>125</sup>l]GLT binding in<br>
human BF and HYP. Data illustrated as percent relative specific binding<br>
of six determinations from three exper BF GAL BF GLT HYP GAL HYP GLI<br> **Fig. 3.** Effect of 100 μM GppNHp on [<sup>125</sup>l]pGAL or [<sup>125</sup>l]GLT binding in<br>
human BF and HYP. Data illustrated as percent relative specific binding<br>
of six determinations from three experim

Framework of  $\mathbf{r}$  and  $\mathbf{r}$  and  $\mathbf{r}$  becomes the specific relative specific binding of six determinations from three experiments (table 1, BF 3, 5 and 9; HYP, 10, 11 and 12) with different membrane preparations **Characterization of**  $[^{125}I]pGAL$  **or**  $[^{125}I]GLT$  **in human tissue with neuropeptides. The specific binding of 40 to 45 pM**  $[^{125}I]GAL$  **or**  $[^{125}I]GLT$  **in the BF was 86**  $\pm$  **6% and 70**  $\pm$  **3%, respectively, in competition Characterization of**  $[^{125}I]pGAL$  **or**  $[^{125}I]GLT$  **in human tissue with neuropeptides. The specific binding of 40 to 45 pM**  $[^{125}I]pGAL$  **or**  $[^{125}I]GLT$  **in the BF was 86**  $\pm$  **6% and 70**  $\pm$  **3%, respectively, in competitio Characterization of**  $[^{126}]$  **pGAL or**  $[^{126}]$  **GLT in human tissue with neuropeptides. The specific binding of 40<br>to 45 pM<sup>** $[^{125}]$ **</sup>pGAL or**  $[^{125}]$ **GLT in the BF was 86**  $\pm$  **6% and<br>70**  $\pm$  **3%, respectively, in competition man tissue with neuropeptides.** The specific binding of 40 to 45 pM  $[^{125}]$ pGAL or  $[^{126}]$ GLT in the BF was 86  $\pm$  6% and 70  $\pm$  3%, respectively, in competition experiments with GAL or GAL chimeric peptides. The spe  $70 \pm 3\%$ , respectively, in competition experiments with GAL or GAL chimeric peptides. The specific binding of 40 to 45 pM  $\left[^{125}I\right]$ pGAL or  $\left[^{125}I\right]$ GLT in the HYP was  $75 \pm 6\%$  and 64  $\pm$ Fig. 3. Effect of 100 *LMM* CHT<br>
Fig. 3. Effect of 100 *μM* GpMHp on [<sup>128</sup>il]GLT brinding in<br>
human BF and HYP. Data illustrated as percent relative specific binding<br>
of six determinations from three experiments (table

ropeptides were selected for testing in the two areas exam-Fo determine spectricity of the GAL receptor, four net repreptides were selected for testing in the two areas examend. Substance P, spantide II, bradykinin (2–9) and cortice tropin-releasing factor were competed at 0.01,



**GLT HYP** licate determinations from one experiment that is<br>representative of three other cases (table 1, BF 1, **Fig. 2.** Saturation of  $[^{125}]$ pGAL and  $[^{125}]$ GLT<br>binding in human BF and HYP (case 5). Varying<br>concentrations of radioligand were incubated **Fig. 2.** Saturation of  $[^{125}]$ JpGAL and  $[^{125}]$ JGLT<br>binding in human BF and HYP (case 5). Varying<br>concentrations of radioligand were incubated<br>with membrane suspensions for 2 hr at room Fig. 2. Saturation of  $[^{125}]$ jpGAL and  $[^{125}]$ GLT<br>binding in human BF and HYP (case 5). Varying<br>concentrations of radioligand were incubated<br>with membrane suspensions for 2 hr at room<br>temperature. Total ( $\bullet$ ), nonspeci binding in numan Br and HYP (case 5). varying<br>
concentrations of radioligand were incubated<br>
with membrane suspensions for 2 hr at room<br>
temperature. Total ( $\bullet$ ), nonspecific ( $\circ$ ), and spe-<br>
cific ( $\bullet$ ) binding were e concentrations or radiologiand were includated<br>with membrane suspensions for 2 hr at room<br>temperature. Total ( $\bullet$ ), nonspecific ( $\Box$ ) binding were estimated as indicated in<br>"Materials and methods." Top,  $[^{125}]$ IpGAL or<br> with membrane suspensions for 2 fir at room<br>temperature. Total ( $\bullet$ ), nonspecific ( $\circ$ ), and specific ( $\bullet$ ) binding were estimated as indicated in<br>"Materials and methods." Top,  $[^{125}$ ljpGAL or<br> $[^{125}$ ljGLT saturatio temperature. I otal ( $\bullet$ ), nonspectric ( $\circ$ ), and specific ( $\bullet$ ) binding were estimated as indicated in "Materials and methods." Top, [<sup>125</sup>l]GAL or [<sup>125</sup>l]GAL 1 or [<sup>125</sup>l]GAL - *K*<sub>D</sub> = 74 pM, B<sub>max</sub> = 31 fmol/mg pro cific ( $\blacksquare$ ) binding were estimated as indicated in<br>"Materials and methods." Top,  $[^{125}][pGAL$  or<br> $[^{125}][qGLT$  saturation binding in the BF. Insets,<br>Scatchard transformations generated by EBDA<br>analysis  $[[^{125}][pGAL - K_p =$ "Materials and methods." Top,  $\lfloor$ <sup>-2-1</sup>]]pGAL or<br>  $\lfloor$ <sup>-25</sup>l]GLT saturation binding in the BF. Insets,<br>
Scatchard transformations generated by EBDA<br>
analysis  $\lfloor$ <sup>125</sup>l]pGAL –  $K_p = 74$  pM,  $B_{max} = 31$ <br>
fmol/mg protein, [<sup>125</sup>][GLI saturation binding in the BF. Insets,<br>Scatchard transformations generated by EBDA<br>analysis ([<sup>125</sup>][GAL -  $K_D = 74$  pM,  $B_{max} = 31$ <br>pM,  $B_{max} = 32$  fmol/mg protein,  $R = .87$ ). Bot-<br>tom, [<sup>125</sup>][GAL or [<sup>125</sup>][GLT Scatchard transformations generated by EBDA<br>analysis ([<sup>125</sup>l]pGAL –  $K_D = 74$  pM, B<sub>max</sub> = 31<br>fmol/mg protein,  $R = .97$ ; [<sup>125</sup>l]GLT –  $K_D = 221$ <br>pM, B<sub>mgx</sub> = 32 fmol/mg protein,  $R = .87$ ). Bot-<br>tom, [<sup>125</sup>l]pGAL or [<sup>125</sup>l] analysis ([<sup>125</sup>l]pGAL -  $K_D = 74$  pM,  $B_{max} = 31$  fmol/mg protein,  $R = .97$ ; [<sup>125</sup>l]GLT -  $K_D = 221$  pM,  $B_{max} = 32$  fmol/mg protein,  $R = .87$ ). Bottom, [<sup>125</sup>l]pGAL or [<sup>125</sup>l]GLT saturation binding in the HYP. Insets, Satur pm,  $B_{mgx} = 32$  imol/mg protein,  $H = .87$ . Bottom,  $[^{125}]$ GAL or  $[^{125}]$ GLT saturation binding in<br>the HYP. Insets, Saturation transformations generated by EBDA analysis  $[^{125}]$ jGAL –  $K_D = 264$ <br>pM,  $B_{mgx} = 125$  fmol/mg p from,  $\Gamma$ <sup>---</sup>ijpoAL of  $\Gamma$ <sup>---</sup>ijoL1 saturation binding in<br>the HYP. Insets, Saturation transformations gen-<br>erated by EBDA analysis ( $\Gamma$ <sup>25</sup>ijpGAL -  $K_D = 264$ <br>pM, B<sub>max</sub> = 125 fmol/mg protein,  $R = .83$ ;<br> $\Gamma$ <sup>125</sup>ijGLT  $[1^{25}$ llGLT –  $K_D$  = 197 pM,  $B_{max}$  = 71.8 fmol/mg<br>protein,  $R$  = .98). Data are mean values of trip-<br>licate determinations from one experiment that is<br>representative of three other cases (table 1, BF 1,<br>2, 5 and 6; HYP

**724** Deecher et al.<br>
ing by 26% in the BF, whereas none of these other neuropep-<br>
tides inhibited the binding of either radioligand.

Because  $[125]pGAL$  and  $[125]GLT$  irreversibly bind to the ing by 26% in the BF, whereas none of these other neuropeptides inhibited the binding of either radioligand.<br>Because  $[{}^{125}I]pGAL$  and  $[{}^{125}I]GLT$  irreversibly bind to the receptor population defined, values for compet ing by 26% in the BF, whereas none of these other neuropeptides inhibited the binding of either radioligand.<br>Because  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  irreversibly bind to the creeptor population defined, values for competit tides inhibited the binding of either radioligand. The the secause  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  irreversibly bind to the compreceptor population defined, values for competition studies the are reported as  $IC_{50}$  rather Because  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  irreversibly bind to t<br>receptor population defined, values for competition stud<br>are reported as  $IC_{50}$  rather than  $K_i$  values. hGAL compet<br>similarly for both radioligands in the HY receptor population defined, values for competition studies the are reported as  $IC_{50}$  rather than  $K_i$  values. hGAL competed radio similarly for both radioligands in the HYP but showed an order of magnitude difference i similarly for both radioligands in the HYP but showed an order of magnitude difference in affinity between radioligands in the BF (fig. 4). GAL and GAL chimeric peptides were competed for the binding of  $[^{125}I]pGAL$  or  $[^$ order of magnitude difference in affinity between radioli-<br>gands in the BF (fig. 4). GAL and GAL chimeric peptides<br>were competed for the binding of  $[^{125}I]pGAL$  or  $[^{125}I]GLT$  in<br>both tissues to determine  $IC_{50}$  values. gands in the BF (fig. 4). GAL and GAL chimeric peptides<br>were competed for the binding of  $[^{125}]$ JpGAL or  $[^{125}]$ JGLT in<br>both tissues to determine  $IC_{50}$  values. (table 2). Peptides  $[^{1}$ <br>competed for the binding of bo were competed for the binding of  $[^{125}]$ pGAL or  $[^{125}]$ GLT in both tissues to determine  $IC_{50}$  values. (table 2). Peptides  $[^{12}$ competed for the binding of both radioligands with similar different values of the HYP, both tissues to determine  $IC_{50}$  values. (table 2). Peptides competed for the binding of both radioligands with similar rank order in the HYP, but in the BF the affinities and rank order differed between radioligands and competed for the binding of both radioligands with similar dif<br>rank order in the HYP, but in the BF the affinities and rank ab<br>order differed between radioligands and regions. GAL and lin<br>GAL chimeric peptides competed an rank order in the HYP, but in the BF the affinities and rank<br>order differed between radioligands and regions. GAL and<br>GAL chimeric peptides competed and were statistically dif-<br>ferent for the binding of  $[^{125}I]pGAL$  in th order differed between radioligands and regions. GAL and GAL chimeric peptides competed and were statistically different for the binding of  $[^{125}]$ pGAL in the BF compared with any other region or radioligand tested. The GAL chimeric peptides competed and were statistically different for the binding of  $[^{125}]$ pGAL in the BF compared with any other region or radioligand tested. The affinities of M35 and M40 were similar for both radioliga ferent for the binding of  $[1^{25}I]pGAL$  in the BF compared with<br>any other region or radioligand tested. The affinities of M35<br>and M40 were similar for both radioligands in the HYP but<br>could not be determined in the BF wit any other region or radioligand tested. The affinities of M35 is<br>and M40 were similar for both radioligands in the HYP but<br>could not be determined in the BF with  $[^{125}I]GLT$ . Also,  $[^{1}$ <br>routine competitions showed M35 and M40 were similar for both radiongalids in the HTF but<br>could not be determined in the BF with  $[^{125}I]GLT$ . Also, [<br>routine competitions showed M35 (1 pM) inhibited ~80% of c<br> $[^{125}I]pGAL$  or  $[^{125}I]GLT$  binding in th routine competitions showed M35  $(1 \text{ pM})$  inhibited ~80% of  $[1^{25}I]pGAL$  or  $[1^{25}I]GLT$  binding in the BF. This finding was unusual because competitions with M35 for hippocampus (pig or rat) or RINm5F cells using  $[1^{2$ unusual because competitions with M35 for hippocampus<br>(pig or rat) or RINm5F cells using  $[^{125}I]pGAL$  competed for<br>the binding over a normal range of activity (data not shown).<br>Kinetics or other neuropeptide receptor int unusual because competitions with M35 for hippocampus that may be due to a high-affinity state of the GAL receptor.<br>(pig or rat) or RINm5F cells using  $[^{125}]$ JpGAL competed for Experiments were designed to address the ir ( $\mu$ g or rat) or itivilior cens using the hydral competed for Exhibities or other neuropeptide receptor interference was considered a pausable explanation for this observation. Because see M35 is the bradykinin chimeric Kinetics or other neuropeptide receptor interference was considered a pausable explanation for this observation. Because M35 is the bradykinin chimeric peptide, interaction with other neuropeptides was investigated. BF me sidered a pausable explanation for this observation. Because<br>M35 is the bradykinin chimeric peptide, interaction with<br>other neuropeptides was investigated. BF membrane homo-<br>ategenates were preincubated for 2 hr with 1  $\$ M35 is the bradykinin chimeric peptide, interaction with in<br>other neuropeptides was investigated. BF membrane homo-<br>genates were preincubated for 2 hr with 1  $\mu$ M fGAL, spantide *et*<br>or bradykinin (2–9) before competitio other neuropeptides was investigated. BF membrane homo-<br>genates were preincubated for 2 hr with 1  $\mu$ M fGAL, spantide *et a*<br>or bradykinin (2–9) before competition of M35 for  $[^{125}I]pGAL$  the<br>binding. If  $[^{125}I]pGAL$  i genates were preincubated for 2 hr with 1  $\mu$ M fGAL, spantide<br>or bradykinin (2–9) before competition of M35 for  $[^{125}I]pGAL$  t<br>binding. If  $[^{125}I]pGAL$  is labeling additional receptors such as<br>substance P or bradykinin or bradykinin (2–9) before competition of M35 for  $\binom{125}{1}pGAL$  th<br>binding. If  $\binom{125}{1}pGAL$  is labeling additional receptors such as<br>substance P or bradykinin, the preincubation step should<br>block these receptors and binding. If [<sup>125</sup>]]pGAL is labeling additional receptors such as substance P or bradykinin, the preincubation step should block these receptors and M35 should compete normally for [<sup>125</sup>]]pGAL binding. No significant effe  $[1^{25}]$ pGAL binding. No significant effect on M35 inhibition of  $[1^{25}]$ pGAL binding was noted in the BF in these experiments, eliminating the possibility of substance P or bradykinin interactions. Because both radioliga  $\binom{125}{126}$  binding was noted in the BF in these experibuand radioligand was removed within the first hour. Sev-<br>ments, eliminating the possibility of substance P or bradykieral additional approaches were undertaken to ments, eliminating the possibility of substance P or bradykinin interactions. Because both radioligands bind irreversibly, the kinetics of M35 was also considered a possible explanation for our reported findings. If the o nin interactions. Because both radioligands bind irreversibly, ci<br>the kinetics of M35 was also considered a possible explana-<br>tion for our reported findings. If the on rate of M35 was<br>faster, then the order of addition of



fig. 4. Competition hGAL using either [<sup>125</sup>i]pGAL or [<sup>125</sup>i]GLT in the whuman BF or HYP. Graph is representative of one experiment ran in triplicate. Values represent the mean and S.D. of percent specific bound at each c **bound at each competition tested.** The method of the repeated at the mean and S.D. of percent specific<br>triplicate. Values represent the mean and S.D. of percent specific<br>bound at each concentration tested. Competitions we Fig. 4. Competition hGAL using either  $[^{125}]\text{pGAL}$  or  $[^{125}]\text{GLT}$  in the human BF or HYP. Graph is representative of one experiment ran in triplicate. Values represent the mean and S.D. of percent specific bound at e

**<sup>724</sup>** Deecher **et al.** Vol. 275 tides inhibited the binding of either radioligand.<br>Because  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  irreversibly bind to the competition for  $[1^{25}I]pGAL$  binding was noted, eliminating receptor population defined, values for compet addition ofeither **['25I]pGAL or M35, respectively, to initiate** *Vol. 275*<br>addition of either  $[^{125}I]pGAL$  or M35, respectively, to initiate<br>the reaction. In these studies, no significant effect on M35<br>competition for  $[^{125}I]pGAL$  binding was noted, eliminating Vol. 275<br>addition of either <sup>[125</sup>I]pGAL or M35, respectively, to initiate<br>the reaction. In these studies, no significant effect on M35<br>competition for <sup>[125</sup>I]pGAL binding was noted, eliminating<br>the possibility of a kinet addition of either  $[{}^{125}I]pGAL$  or M35, respectively, to initiate the reaction. In these studies, no significant effect on M35 competition for  $[{}^{125}I]pGAL$  binding was noted, eliminating the possibility of a kinetic d **radioligand.**

# **Discussion**

dioligand.<br> **Discussion**<br>
The present findings indicate that  $[^{125}I]pGAL$  and<br>  $^{25}I]GLT$  are slowly reversible high-affinity agonists that **Discussion**<br>
The present findings indicate that  $[^{125}I]pGAL$  and<br>  $[^{125}I]GLT$  are slowly reversible high-affinity agonists that<br>
differentiate GAL receptor subtypes in the BF and HYP. The<br>
ability to generate a large nu The present findings indicate that  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  are slowly reversible high-affinity agonists that differentiate GAL receptor subtypes in the BF and HYP. The ability to generate a large number of brain sam  $[1^{25}]$ GLT are slowly reversible high-affinity agonists the differentiate GAL receptor subtypes in the BF and HYP. The ability to generate a large number of brain samples himited the scope of the present study, and there differentiate GAL receptor subtypes in the BF and HYP. The ability to generate a large number of brain samples has limited the scope of the present study, and therefore certain questions regarding receptor subtypes, radiol investigation. mited the scope of the present study, and therefore certain<br>restions regarding receptor subtypes, radioligand specific-<br> $\alpha$  and GAL chimeric peptide interactions require further<br>vestigation.<br>The results of the kinetic ex questions regarding receptor subtypes, radioligand specificity and GAL chimeric peptide interactions require further investigation.<br>The results of the kinetic experiments showed that both  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  irr

cific binding could b<br>branes with acetic aci<br>removed all bound<br>washed and reused in<br>binding was restored.<br>These results indica anes with acetic acid after preincubation with  $[^{125}I]pGAL$  moved all bound radioligand. These membranes were ashed and reused in binding experiments, and the specific mding was restored.<br>These results indicate that  $[^{1$ ity and GAL chimeric peptide interactions require further<br>investigation.<br>The results of the kinetic experiments showed that both<br> $[{}^{125}I]pGAL$  and  $[{}^{125}I]GLT$  irreversibly bind to the GAL re-<br>ceptor after association, investigation.<br>The results of the kinetic experiments showed that both  $[{}^{125}I]pGAL$  and  $[{}^{125}I]GLT$  irreversibly bind to the GAL receptor after association, even in the presence of  $>1000$ -fold excess of unlabeled pep The results of the kinetic experiments showed that both  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  irreversibly bind to the GAL receptor after association, even in the presence of  $>1000$ -fold excess of unlabeled peptide. This was an  $[{}^{125}]$ JpGAL and  $[{}^{125}]$ JGLT irreversibly bind to the GAL receptor after association, even in the presence of >1000-fold excess of unlabeled peptide. This was an unexpected finding that may be due to a high-affinity s ceptor after association, even in the presence of >1000-101d<br>excess of unlabeled peptide. This was an unexpected finding<br>that may be due to a high-affinity state of the GAL receptor.<br>Experiments were designed to address th that may be due to a high-affinity state of the GAL receptor.<br>Experiments were designed to address the irreversibility or<br>slowly reversible action of  $[^{125}I]pGAL$  or  $[^{125}I]GLT$  binding<br>for GAL receptors based on prior i Experiments were designed to address the irreversibility<br>slowly reversible action of  $[^{125}]$  pGAL or  $[^{125}]$  JGLT bindi<br>for GAL receptors based on prior information. Several is<br>searchers reported that GAL elicits its bi slowly reversible action of  $[{}^{125}I]pGAL$  or  $[{}^{125}I]GLT$  binding<br>for GAL receptors based on prior information. Several re<br>searchers reported that GAL elicits its biological effects by<br>interacting with membrane-bound re for GAL receptors based on prior information. Several researchers reported that GAL elicits its biological effects by interacting with membrane-bound receptors that are associated with  $G_i$  proteins (Amiranoff *et al.*, 1 searchers reported that GAL elicits its biological effects by<br>interacting with membrane-bound receptors that are associ-<br>ated with G<sub>i</sub> proteins (Amiranoff *et al.*, 1988; Lagny-Pourmir<br>*et al.*, 1989). If both  $[^{125}]$ Jp interacting with membrane-bound receptors that are associated with  $G_i$  proteins (Amiranoff *et al.*, 1988; Lagny-Pourmir *et al.*, 1989). If both  $[^{125}]$  pGAL and  $[^{125}]$  GLT are agonists that bind to high-affinity GAL ated with G<sub>i</sub> proteins (Amiranoff *et al.*, 1988; Lagny-Pourmir *et al.*, 1989). If both  $[{}^{125}I]pGAL$  and  $[{}^{125}I]GLT$  are agonists that bind to high-affinity GAL receptors, forming a stable ternary complex (De Lean *e* that bind to high-affinity GAL receptors, forming a stable<br>ternary complex (De Lean *et al.*, 1980), then the presence of<br>GppNHp should dissociate the G protein from the receptor<br>and reduce agonist binding. Routine dissoc that bind to high-affinity GAL receptors, forming a stable<br>ternary complex (De Lean *et al.*, 1980), then the presence of<br>GppNHp should dissociate the G protein from the receptor<br>and reduce agonist binding. Routine dissoc ternary complex (De Lean *et al.*, 1980), then the presence of GppNHp should dissociate the G protein from the receptor and reduce agonist binding. Routine dissociation experiments were done in the presence of GppNHp, and GppNHp should dissociate the G protein from the recept<br>and reduce agonist binding. Routine dissociation exper<br>ments were done in the presence of GppNHp, and <20% of<br>bound radioligand was removed within the first hour. Seve and reduce agonist binding. Routine dissociation experiments were done in the presence of GppNHp, and  $\langle 20\%$  of bound radioligand was removed within the first hour. Several additional approaches were undertaken to try ments were done in the presence of GppNHp, and  $\langle 20\%$  of bound radioligand was removed within the first hour. Several additional approaches were undertaken to try to dissociate the radioligands from the GAL receptor. B eral additional approaches were undertaken to try to disso-<br>ciate the radioligands from the GAL receptor. BF membranes<br>were repeatedly washed after preincubation with  $[1^{25}I]p-$ <br>GAL, and the amount of bound radioligand ciate the radioligands from the GAL receptor. BF membranes<br>were repeatedly washed after preincubation with  $[^{125}I]p$ -GAL, and the amount of bound radioligand was measured.<br>Readily reversible ligands can be removed with t were repeatedly washed after preincubation with  $[1^{25}I]$ <br>GAL, and the amount of bound radioligand was measure<br>Readily reversible ligands can be removed with this was<br>technique (Deecher *et al.*, 1991, 1992). No reductio GAL, and the amount of bound radioligand was measured.<br>Readily reversible ligands can be removed with this wash<br>technique (Deecher *et al.*, 1991, 1992). No reduction in spe-<br>cific binding could be measured. Treatment of Readily reversible ligands can be removed with this wash<br>technique (Deecher *et al.*, 1991, 1992). No reduction in spe-<br>cific binding could be measured. Treatment of BF mem-<br>branes with acetic acid after preincubation with

removed all bound radioligand. These membranes were<br>washed and reused in binding experiments, and the specific<br>binding was restored.<br>These results indicate that  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  bind<br>very tightly to the recep washed and reused in binding experiments, and the specific<br>binding was restored.<br>These results indicate that  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  bind<br>very tightly to the receptor but that their interaction does not<br>change the c binding was restored.<br>These results indicate that  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  bind<br>very tightly to the receptor but that their interaction does not<br>change the conformation of the GAL receptor. Data on the<br>irreversibili These results indicate that  $[^{125}][\text{BGAL}$  and  $[^{125}][\text{GLT}$  bind<br>very tightly to the receptor but that their interaction does not<br>change the conformation of the GAL receptor. Data on the<br>irreversibility or slowly reve [<sup>125</sup>I]GLT binding have been shown in other reports, although not clearly identified. Servin *et al.* (1987) performed dissociation kinetics in the presence of 100 nM GAL with  $[1^{25}I]pGAL$  in rat brain and reported a di  $[{}^{125}]$ GLT binding have been shown in other reports, although not clearly identified. Servin *et al.* (1987) performed dissociation kinetics in the presence of 100 nM GAL with  $[{}^{125}]$ JpGAL in rat brain and reported a  $\frac{1}{2}$   $\frac{1}{2}$   $\frac{1}{2}$  **I** though not clearly identified. Servin *et al.* (1987) performed<br>dissociation kinetics in the presence of 100 nM GAL with<br> $\frac{12^{25}}{10^{-12}}$   $\frac{1}{10^{-10}}$   $\frac{10^{8}}{10^{-8}}$   $\frac{10^{6}}{10^{-8}}$ **hough not clearly identified. Servin** *et al.* **(1987) performed<br>dissociation kinetics in the presence of 100 nM GAL with<br>** $[1^{25}I]pGAL$  **in rat brain and reported a dissociation constant<br>of**  $K_{-1} = 0.039$  **min<sup>-1</sup>, even thou** of  $K_{-1} = 0.039 \text{ min}^{-1}$ , even though after 1 and 2 hr, 50% and 25% of the specific binding remained, respectively. Other reports have noted similar  $[^{125}]$  pGAL kinetics in agreement to those reported in the present st reports have noted similar  $\left[^{125}]\text{pGAL}$  kinetics in agreement<br>to those reported in the present study. Sharp *et al.*, (1989),<br>with RINm5F cells, reported that the kinetics were partially<br>reversible in the presence of reversible in the presence of 800 pM  $[$ <sup>125</sup>I]pGAL, with 40% of to those reported in the present study. Sharp *et al.*, (1989), with RINm5F cells, reported that the kinetics were partially reversible in the presence of 800 pM  $[^{125}]$ pGAL, with 40% of the ligand still bound after 2 hr with RINm5F cells, reported that the kinetics were partially reversible in the presence of 800 pM  $[^{125}]$ pGAL, with 40% of the ligand still bound after 2 hr. Lagny-Pourmir *et al.* (1989) demonstrated that only 30% of  $[^$ 

1995<br>TABLE 2<br>**Competition of** G Competition of GAL and GAL chimeric peptides using [<sup>125</sup>i]GAL or GLT in BF or HYP provides evidence to suggest the existence of<br>Competition of GAL and GAL chimeric peptides using [<sup>125</sup>i]GAL or GLT in BF or HYP provides e

1995<br>
TABLE 2<br>
Competition of GAL and GAL chimeric peptides using  $[^{125}I]GAL$  or GLT in BF or HYP provides evidence to suggest the existence of<br>
different GAL receptor subtypes in the human brain<br>
Results are expressed a ent GAL receptor subtypes in the human brain<br>s are expressed as mean  $\pm$  S.E.M. of the IC<sub>so</sub> (pM) corresponding to three independent experiments. Values for IC<sub>so</sub> were estimated using the algorithm EBDA.<br>In the actor f



**P**  $\le$  .05 vs. all groups within the same competitor.<br> **P**  $\le$  .05 vs. HYP using both radioligands.<br> **P**  $\le$  .05 vs. BF/[<sup>125</sup>l]GAL.<br> **P**  $\le$  .05 vs. BF/[<sup>125</sup>l]GAL ranking of competitors in which the minimal dose of in radioligands.<br>
and intervel in the minimal dose of competitor added exhibited maximal<br>
intervel in the minimal dose of competitor added exhibited maximal<br>
intervel in the minimal dose of competitor added exhibited maxi

 $BF/$ <sup>125</sup>IJGLT ranking of competitors: M35 = M40 = C7 < pGAL = rGAL = hGAL < fGAL = GLT.

**EFILES INTERFERIGAL ranking of competitors:** M35 < pGAL <<br>BFILES IRAL Tranking of competitors: M35 = M40 = C<br>HYPI<sup>125</sup>IIGAL ranking of competitors: M35 = M40 = C<br>HYPI<sup>125</sup>IIGAL ranking of competitors: rGAL = pGAL =<br> $\frac{1$  $BF/I^{125}$ []GLT ranking of competitors: M35 = M40 =<br>  $HYP/I^{125}$ []GAL ranking of competitors: rGAL = pGAL<br>
>50% of the specific binding of  $[1^{25}I]pGAL$  was removed<br>
within 5 min in the presence of 10  $\mu$ M GppNHp. It is po **the the three transition** of competitors: iGAL = pGAL =<br>  $HYP(T^{25}I]GLT$  ranking of competitors: iGAL = pGAL =<br>  $>50\%$  of the specific binding of  $[1^{25}I]pGAL$  was removed in<br>
within 5 min in the presence of 10 μM GppNHp.  $>50\%$  of the specific binding of  $[^{125}]$ pGAL was removithin 5 min in the presence of  $10 \mu M$  GppNHp. It is possibile that the high-affinity state of the GAL receptor population responsible for the irreversible portion within 5 min in the presence of 10  $\mu$ M GppNHp. It is possible<br>that the high-affinity state of the GAL receptor population is<br>tate of the GAL receptor population. The tight agonist bind-<br>responsible for the irreversible within 5 min in the presence of  $10 \mu M$  GppNHp. It is possible show<br>that the high-affinity state of the GAL receptor population is state<br>responsible for the irreversible portion of the binding. Previ-<br>jous investigators m that the high-affinity state of the GAL receptor population is state<br>responsible for the irreversible portion of the binding. Previ-<br>jous investigators may be reporting the reversible binding<br>portion of the low-affinity st responsible for tl<br>ous investigator<br>portion of the lov<br>different subtyp<br>species specific.<br>Although our i **Although our findings of irreversible kinetics are unusual other neuropeptide, endothelin (Hocher** *et al.***, 1992)<br>Although our findings of irreversible kinetics are unusual<br>nother neuropeptide, endothelin (Hocher** *et al.* portion of the low-affinity state of the receptor or studying a different subtype of the GAL receptor that is region and species specific.<br>Although our findings of irreversible kinetics are unusual, another neuropeptide, e

different subtype of the GAL receptor that is region and species specific.<br>Although our findings of irreversible kinetics are unusual,<br>another neuropeptide, endothelin (Hocher *et al.*, 1992;<br>Watakabe *et al.*, 1992; Wu-Wo species specific.<br>Although our findings of irreversible kinetics are unusual,<br>another neuropeptide, endothelin (Hocher *et al.*, 1992;<br>Watakabe *et al.*, 1992; Wu-Wong *et al.*, 1993), has similar<br>kinetic characteristics. Atthough our minings of freversible kinetics are unusual,<br>
another neuropeptide, endothelin (Hocher et al., 1992;<br>
Watakabe et al., 1992; Wu-Wong et al., 1993), has similar n<br>
kinetic characteristics. Severne et al. (1987) Watakabe *et al.*, 1992; Wu-Wong *et al.*, 1993), has similar nists<br>kinetic characteristics. Severne *et al.* (1987) proposed a the-<br>ory regarding high-affinity agonists, termed "tight agonist from<br>binding." This theory st kinetic characteristics. Severne *et al.* (1987) proposed a theory regarding high-affinity agonists, termed "tight agonist binding." This theory states that when agonists bind, a stable receptor ligand complex is formed th ory regarding high-affinity agonists, termed "tight agonis<br>binding." This theory states that when agonists bind, a stable<br>receptor ligand complex is formed that locks the agonist i<br>the binding pocket for as long as the rec binding." This theory states that when agonists bind, a stable receptor ligand complex is formed that locks the agonist in the binding pocket for as long as the receptor is functionally associated with their perspective G the binding pocket for as long as the receptor is functionally the HYP. A recent article characterizing GAL receptors in associated with their perspective G proteins. This tight ago-<br>nist binding occurs in only a portion the binding pocket for as long as the receptor is functionally the associated with their perspective G proteins. This tight agonist binding occurs in only a portion of the receptor population (high affinity) and is common associated with their perspective G proteins. This tight agonist binding occurs in only a portion of the receptor population (high affinity) and is commonly characterized by slowly reversible kinetics. This theory fits we mist binding occurs in only a portion of the receptor popula-<br>tion (high affinity) and is commonly characterized by slowly<br>wireversible kinetics. This theory fits well with the interaction<br>of  $[^{125}I]pGAL$  and  $[^{125}I]GLT$ tion (high affinity) and is commonly characterized by slowly<br>reversible kinetics. This theory fits well with the interaction<br>of  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  binding in the BF and HYP,<br>with the exception of GppNHp not ful reversible kinetics. This theory fits well with the interaction the GA of  $[1^{25}I]pGAL$  and  $[1^{25}I]GLT$  binding in the BF and HYP, rently with the exception of GppNHp not fully dissociating the studies radioligands in our of  $[^{125}]$ pGAL and  $[^{125}]$ GLT binding in the BF and HYP, rent<br>with the exception of GppNHp not fully dissociating the stude<br>radioligands in our kinetic experiments. It is probable that In<br>the tight agonist binding forms with the exception of GppNHp not fully dissociating the stude radioligands in our kinetic experiments. It is probable that In the tight agonist binding forms a very stable receptor G ber of protein complex (ternary comple radioligands in our kinetic experiments. It is probable that<br>the tight agonist binding forms a very stable receptor G ber<br>protein complex (ternary complex) that is not affected by the<br>the<br>presence of GppNHp after saturatio the tight agonist binding forms a very stable receptor protein complex (ternary complex) that is not affected by the presence of GppNHp after saturation. If GppNHp were addefore saturation, then binding of either  $[^{125}I]$ protein complex (ternary complex) that is not affected by the<br>presence of GppNHp after saturation. If GppNHp were added<br>before saturation, then binding of either  $[^{125}]$ JpGAL or<br> $[^{125}]$ JGLT was reversed. Preincubation of presence of GppNHp after saturation. If GppNHp were added ma<br>before saturation, then binding of either  $[^{125}I]pGAL$  or pop<br> $[^{125}I]GLT$  was reversed. Preincubation of BF or HYP mem-<br>branes with GppNHp reduced the specifi <sup>[125</sup>]]GLT was reversed. Preincubation of BF or HYP membranes with GppNHp reduced the specific binding of bot radioligands, indicating that a major portion of the binding is associated with G proteins and that both radiol branes with GppNHp reduced the specific binding of bot radioligands, indicating that a major portion of the binding associated with G proteins and that both radioligands as agonist. However, a portion of the specific bind radioligands, indicating<br>associated with G prot<br>agonist. However, a pologal was not GppNHp<br>ated with G proteins.<br>Based on our observi sociated with G proteins and that both radioligands are<br>
sociated with G proteins of the specific binding of  $[1^{25}]$  p-<br>
AL was not GppNHp sensitive and therefore is not associ-<br>
ed with G proteins.<br>
Based on our observat

agonist. However, a portion of the specific binding of  $[1^{25}]$  p-<br>GAL was not GppNHp sensitive and therefore is not associ-<br>ated with G proteins. (A<br>Based on our observations, we propose two affinity states am<br>for the GA GAL was not GppNHp sensitive and therefore is not associated with G proteins.<br>Based on our observations, we propose two affinity states<br>for the GAL receptor population that was identified with<br> $[^{125}I]pGAL$  labeling: a hig ated with G proteins.<br>
Based on our observations, we propose two affinity states<br>
for the GAL receptor population that was identified with<br>  $[1^{25}I]pGAL$  labeling: a high-affinity and a low-affinity state.<br>
Gu *et al.* (19 Based on our observations, we propose two affinity states<br>for the GAL receptor population that was identified with<br> $G^{125}$ I]pGAL labeling: a high-affinity and a low-affinity state.<br>Gu *et al.* (1994) reported evidence of for the GAL receptor population that was identified with  $G_l^{125}I]pGAL$  labeling: a high-affinity and a low-affinity state. pe<br>Gu *et al.* (1994) reported evidence of high- and low-affinity itis<br>states of the GAL receptor <sup>[125</sup>]]pGAL labeling: a high-affinity and a low-affinity state Gu *et al.* (1994) reported evidence of high- and low-affinity states of the GAL receptor in guinea pig stomach smootl muscle cells, which is in agreement wit

 $\therefore$  GAL = C7 = hGAL < M40 = GLT < fGAL.<br>  $\le$  GAL = C7 = hGAL = hGAL < fGAL = GLT.<br>
L = hGAL < M35 = M40 = fGAL < C7 = GLT.<br>
L = hGAL < M35 = M40 = fGAL < C7 = GLT.<br>
L = hGAL < M40 = M35 = C7 = fGAL < GLT.<br>
ing theory, showed reversible kinetics is associated with the low-affinity<br>showed reversible kinetics is associated with the low-affinity<br>showed reversible kinetics is associated with the low-affinity<br>state of the GAL receptor popula Let  $\text{H} = \text{HGAL} < \text{M} = \text{HGAL} < \text{GIT}$ .<br>
Let  $\text{HGAL} < \text{M} = \text{HGAL} < \text{GIT}$ .<br>  $\text{H} = \text{HGAL} < \text{M} = \text{HGAL} < \text{GLT}$ .<br>  $\text{H} = \text{HGAL} \times \text{M} = \text{HGAL} \times \text{GIT}$ .<br>  $\text{H} = \text{HGAL} \times \text{M} = \text{HGAL} \times \text{GIT}$ .<br>  $\text{H} = \text{HGAL} \times \text{M$ ing theory, that the 25% of the binding in our studies that<br>showed reversible kinetics is associated with the low-affinity<br>state of the GAL receptor population. The tight agonist bind-<br>ing theory fits well with our data w showed reversible kinetics is associated with the low-affinity<br>state of the GAL receptor population. The tight agonist bind-<br>ing theory fits well with our data with  $[^{125}I]pGAL$  labeling<br>but not with  $[^{125}I]GLT$  labeling state of the GAL receptor population. The tight agonist bind-<br>ing theory fits well with our data with  $[^{125}I]pGAL$  labeling<br>but not with  $[^{125}I]GLT$  labeling because all of the binding was<br>GppNHp dependent. In contrast, but not with [<sup>125</sup>I]GLT labeling because all of the binding was GppNHp dependent. In contrast, a portion of the [<sup>125</sup>I]pGAL binding was GppNHp insensitive, yet both radioligands showed similar proportional reversible kin but not with  $[^{125}]$ GLT labeling because all of the binding was GppNHp dependent. In contrast, a portion of the  $[^{125}]$ pGAL binding was GppNHp insensitive, yet both radioligands showed similar proportional reversible kin GppNHp dependent. In contrast, a portion of the [<sup>125</sup>I]pGAL binding was GppNHp insensitive, yet both radioligands showed similar proportional reversible kinetics, which is indicative of low-affinity binding. Our findings binding was GppNHp insensitive, yet both ra<br>showed similar proportional reversible kinetics, w<br>dicative of low-affinity binding. Our findings in co<br>with this theory suggest that both radioligands a<br>mists because both ligan owed similar proportional reversible kinetics, which is incative of low-affinity binding. Our findings in conjunction<br>th this theory suggest that both radioligands act as ago-<br>sts because both ligands are GppNHp sensitive. dicative of low-affinity binding. Our findings in conjunction<br>with this theory suggest that both radioligands act as ago-<br>nists because both ligands are GppNHp sensitive.<br>Rosenthal analysis on data taken at assumed saturat

with this theory suggest that both radioligands act as agonists because both ligands are GppNHp sensitive.<br>Rosenthal analysis on data taken at assumed saturation<br>from binding experiments in the BF and HYP reveals that<br>both nists because both ligands are GppNHp sensitive.<br>
Rosenthal analysis on data taken at assumed saturation<br>
from binding experiments in the BF and HYP reveals that<br>
both radioligands bind with high affinity to GAL receptors<br> Rosenthal analysis on data taken at assumed saturation<br>from binding experiments in the BF and HYP reveals that<br>both radioligands bind with high affinity to GAL receptors<br>and that the greatest number of binding sites were from binding experiments in the BF and HYP reveals that both radioligands bind with high affinity to GAL receptors and that the greatest number of binding sites were found in the HYP. A recent article characterizing GAL r both radioligands bind with high affinity to GAL receptors<br>and that the greatest number of binding sites were found in<br>the HYP. A recent article characterizing GAL receptors in<br>human HYP with  $[1^{25}I]pGAL$  reported slowly and that the greatest number of binding sites were found in<br>the HYP. A recent article characterizing GAL receptors in<br>human HYP with  $[^{125}]$ pGAL reported slowly reversible ki-<br>netics and similar binding affinities, which the HYP. A recent article characterizing GAL receptors in<br>human HYP with  $[1^{25}]$  pGAL reported slowly reversible ki-<br>netics and similar binding affinities, which is in agreement<br>with our study, but reported 4-fold greater studies were taken from approximately the same age group. with our study, but reported 4-fold greater binding density of<br>the GAL receptor (Lorinet *et al.*, 1994). This finding is cur-<br>rently unexplainable because the tissue samples in both<br>studies were taken from approximately

In the BF,  $[$ <sup>125</sup>I]pGAL and  $[$ <sup>125</sup>I]GLT label the same numrently unexplainable because the tissue samples in both<br>studies were taken from approximately the same age group.<br>In the BF,  $[^{125}I]DGAL$  and  $[^{125}I]GLT$  label the same num-<br>ber of sites, with  $[^{125}I]DGAL$  having the h studies were taken from approximately the same age group.<br>In the BF,  $[{}^{125}I]pGAL$  and  $[{}^{125}I]GLT$  label the same num-<br>ber of sites, with  $[{}^{125}I]pGAL$  having the highest affinity for<br>the receptor population in this t In the BF,  $[{}^{125}]$ JpGAL and  $[{}^{125}]$ JGLT label the same num<br>ber of sites, with  $[{}^{125}]$ JpGAL having the highest affinity f<br>the receptor population in this tissue. Although these da<br>may suggest that both radioligands l ber of sites, with  $[^{125}]$ pGAL having the highest affinity for<br>the receptor population in this tissue. Although these data<br>may suggest that both radioligands label the same receptor<br>population in the BF, the competition the receptor population in this tissue. Although these dat<br>may suggest that both radioligands label the same recepto<br>population in the BF, the competition studies indicate other<br>wise. Also, our data demonstrate that  $[1^{25$ may suggest that both radioligands label the same receptor population in the BF, the competition studies indicate otherwise. Also, our data demonstrate that  $[1^{25}I]GAL$  labels 50% more sites than  $[1^{25}I]GLT$  in the HYP, population in the BF, the competition studies indicate otherwise. Also, our data demonstrate that  $[^{125}I]pGAL$  labels 50% more sites than  $[^{125}I]GLT$  in the HYP, suggesting an additional receptor population or affinity se. Also, our data demonstrate that  $[^{125}]$ pGAL labels 50%<br>ore sites than  $[^{125}]$ GLT in the HYP, suggesting an addi-<br>onal receptor population or affinity state of the GAL recep-<br>r that is not recognized by  $[^{125}]$ GLT.<br>S

more sites than  $[1^{25}I]GLT$  in the HYP, suggesting an additional receptor population or affinity state of the GAL receptor that is not recognized by  $[1^{25}I]GLT$ .<br>Studies have shown that the first 13 amino acids in the G tional receptor population or affinity state of the GAL receptor that is not recognized by  $[1^{25}I]GLT$ .<br>Studies have shown that the first 13 amino acids in the GAL sequence are essential for binding to the GAL receptor ( tor that is not recognized by  $[^{125}I]GLT$ .<br>Studies have shown that the first 13 amino acids in the<br>GAL sequence are essential for binding to the GAL receptor<br>(Amiranoff *et al.*, 1989); Fisone *et al.*, 1989). These firs Studies have shown that the first 13 amino acids in the GAL sequence are essential for binding to the GAL receptor (Amiranoff *et al.*, 1989; Fisone *et al.*, 1989). These first 13 amino acids are 100% homologous between s GAL sequence are essential for binding to the GAL recepto<br>(Amiranoff *et al.*, 1989; Fisone *et al.*, 1989). These first 1<br>amino acids are 100% homologous between species in th<br>GAL peptides (Tatemoto *et al.*, 1983) and t (Amiranoff *et al.*, 1989; Fisone *et al.*, 1989). These first 13 amino acids are 100% homologous between species in the GAL peptides (Tatemoto *et al.*, 1983) and the GAL chimeric peptides (Langel *et al.*, 1992) tested amino acids are 100% homologous between species in GAL peptides (Tatemoto *et al.*, 1983) and the GAL chime peptides (Langel *et al.*, 1992) tested in this study. The affities of the GAL peptides were determined with  $\binom{$ GAL peptides (Tatemoto *et al.*, 1983) and the GAL chimeric<br>peptides (Langel *et al.*, 1992) tested in this study. The affin-<br>ities of the GAL peptides were determined with  $[1^{25}I]pGAL$ <br>or  $[1^{25}I]GLT$  to discern any dif peptides (Langel *et al.*, 1992) tested in this study. The affinities of the GAL peptides were determined with  $[^{125}]$ GAL or  $[^{125}]$ GLT to discern any differences in binding interactions of either radioligand in the BF o

**726 Deecher et al.**<br>showed a statistically significant lower affinity for  $[^{125}I]$ <br>GAL binding. Comparison of  $IC_{50}$  values from competition **726 Deecher et al.**<br>showed a statistically significant lower affinity for  $[^{125}I]p-$  GAL binding. Comparison of IC<sub>50</sub> values from competition s<br>studies in the HYP shows that all peptides compete similarly a **726 Deecher et al.**<br>showed a statistically significant lower affinity for  $[^{125}I]p-$  GAL binding. Comparison of  $IC_{50}$  values from competition studies in the HYP shows that all peptides compete similarly ain rank or showed a statistically significant lower affinity for  $[^{125}]$ p-<br>GAL binding. Comparison of  $IC_{50}$  values from competition<br>studies in the HYP shows that all peptides compete similarly<br>in rank order for  $[^{125}]$ pGAL and  $[^$ showed a statistically significant lower affinity for  $[{}^{125}I]p$  GAL binding. Comparison of  $IC_{50}$  values from competition studies in the HYP shows that all peptides compete similarly in rank order for  $[{}^{125}I]pGAL$  a GAL binding. Comparison of  $IC_{50}$  values from competition studies in the HYP shows that all peptides compete similarly a<br>in rank order for  $[^{125}I]pGAL$  and  $[^{125}I]GLT$  binding. In con-<br>trast, all peptides, with the exc studies in the HYP shows that all peptides compete similarly available in rank order for  $[^{125}]$  pGAL and  $[^{125}]$ GLT binding. In contrast, all peptides, with the exception of pGAL and C7, compreted differently for  $[^{12$ in rank order for  $[^{125}]$ **J**GAL and  $[^{125}]$ GLT binding. In contrast, all peptides, with the exception of pGAL and C7, competed differently for  $[^{125}]$ **pGAL** in the BF. The varying affinities noted in the competition st trast, all peptides, with the exception of pGAL and C7, com-<br>
peted differently for  $[1^{25}I]pGAL$  in the BF. The varying af-<br>
finities noted in the competition studies and the results from cological treatment strategy for tested. finities noted in the competition studies and the results from<br>the saturation experiments suggest at least two receptor<br>subtypes depicted by the choice of radioligand and region<br>tested.<br>The results from the competition stu

subtypes depicted by the choice of radioligand and region will be useful when characterizing the receptor pharmacol-<br>tested.<br>The results from the competition studies of the GAL chi-<br>meric peptides M35, M40 and C7 were unus meric peptides M35, M40 and C7 were unusual in the BF ogy<br>
The results from the competition studies of the GAL chi-<br>
meric peptides M35, M40 and C7 were unusual in the BF<br>
homogenates. Due to lack of human tissue in these regions,<br>
additional studies on only one GAL chimeric The results from the competition studies of the GAL chimeric peptides M35, M40 and C7 were unusual in the BF homogenates. Due to lack of human tissue in these regions, additional studies on only one GAL chimeric peptide co meric peptides M35, M40 and C7 were unusual in the BF ahomogenates. Due to lack of human tissue in these regions, additional studies on only one GAL chimeric peptide could be lenvestigated. M35, the bradykinin chimera, inh additional studies on only one GAL chimeric peptide could be<br>investigated. M35, the bradykinin chimera, inhibited at least<br>80% of the binding of either radioligand at low concentrations<br>from human BF and HYP, pig<br>finding, investigated. M35, the bradykinin chimera, inhibited at lea<br>80% of the binding of either radioligand at low concentration<br>(1 pM) in the BF. To ensure that this was a substantiate<br>finding, receptor preparations from human B 80% of the binding of either radioligand at low concentrations (1 pM) in the BF. To ensure that this was a substantiated finding, receptor preparations from human BF and HYP, pig and rat hippocampus and RINm5F were perfor (1 pM) in the BF. To ensure that this was a substantiate finding, receptor preparations from human BF and HYP, p and rat hippocampus and RINm5F were performed in parallel experiments in the presence of competing concentra finding, receptor preparations from human BF and HYP, pig<br>and rat hippocampus and RINm5F were performed in paral-<br>lel experiments in the presence of competing concentrations<br>of M35 for  $[^{125}I]pGAL$  binding. The  $IC_{50}$  v and rat hippocampus and RINm5F were performed in paral-<br>lel experiments in the presence of competing concentrations<br>of M35 for  $[^{125}]$ pGAL binding. The IC<sub>50</sub> values were deter-<br>mined in the picomolar range, with the exce lel experiments in the presence of competing concentrations<br>of M35 for  $[^{125}]$ pGAL binding. The  $IC_{50}$  values were deter-<br>mined in the picomolar range, with the exception of the<br>human BF homogenates. These observations of M35 for  $[^{125}]$ pGAL binding. The  $IC_{50}$  values were determined in the picomolar range, with the exception of the human BF homogenates. These observations suggest that the present findings are accurate. Kinetics or ot mined in the picomolar range, with the exception of the AMTRANOFF, B., LORINET, A., LAGNY-POURMIR, I. AND LABURTHE, M.: Mechanism of<br>
the present findings are accurate. Kinetics or other neu-<br>
ropeptide interference was be human BF homogenates. These observations suggest that<br>the present findings are accurate. Kinetics or other neu-<br>ropeptide interference was believed to be a possible explana-<br>tion for this finding. In experiments to address the present findings are accurate. Kinetics or other neu-<br>ropeptide interference was believed to be a possible explana-<br>tion for this finding. In experiments to address neuropeptide<br>specificity, several neuropeptides were ropeptide interference was believed to be a possible explana-<br>tion for this finding. In experiments to address neuropeptide<br>specificity, several neuropeptides were competed for the<br>binding of  $[^{125}I]GAL$  or  $[^{125}I]GLT$ . specificity, several neuropeptides were competed for the binding of  $[^{125}]$ pGAL or  $[^{125}]$ GLT. None of the peptides nal tested inhibited the binding of either radioligand except for substance P (1  $\mu$ M), which inhibite binding of  $[1^{25}I]pGAL$  or  $[1^{25}I]GLT$ . None of the peptides<br>tested inhibited the binding of either radioligand except for<br>substance P (1  $\mu$ M), which inhibited  $[1^{25}I]pGAL$  binding by<br> $26\%$ . BF receptor preparations substance  $P (1 \mu M)$ , which inhibited  $[1^{25}]$  pGAL binding by<br>
26%. BF receptor preparations were also preincubated with<br>  $2^{20}$  before routine binding<br>
FGAL, spantide or bradykinin (2-9) before routine binding<br>
FGAL, sp were done to determine whether the complete inhibition of<br>
<sup>BYLUND</sup>, D. B. AND YAMAMURA, H. 1.: Methods for receptor banding. *In* Neurotransmitter Receptor analysis, pp. 1–36, Raven Press, NY, 1990.<br>
<sup>125</sup>I]pGAL binding b fGAL, spantide or bradykinin (2-9) before routine binding fGAL, spantide or bradykinin (2–9) before routine binding<br>experiments competing M35 were run. These experiments mole<br>were done to determine whether the complete inhibition of<br> $[^{125}I]pGAL$  binding by M35 was due to other experiments competing M35 were run. These experime<br>were done to determine whether the complete inhibition<br> $[1^{25}I]pGAL$  binding by M35 was due to other receptor int<br>action. These experiments showed only a  $10\%$  reduction were done to determine whether the complete inhibition of  $[{}^{125}]$  pGAL binding by M35 was due to other receptor interaction. These experiments showed only a 10% reduction on the inhibitory effect of M35 for  $[{}^{125}]$  pGA <sup>[125</sup>I]pGAL binding by<br>action. These experime<br>the inhibitory effect of<br>ing that M35 is prima<br>labeled by <sup>[125</sup>I]pGAL.<br>The other possible ex tion. These experiments showed only a 10% reduction on<br>
e inhibitory effect of M35 for  $[{}^{125}I]pGAL$  binding, indicat-<br>
g that M35 is primarily interacting with GAL receptors<br>
beled by  $[{}^{125}I]pGAL$ .<br>
The other possible

the inhibitory effect of M35 for  $[^{125}]$ pGAL binding, indicat-<br>ing that M35 is primarily interacting with GAL receptors<br>is the complete inhibition<br>absence: A hypothesis for the role of galanin in accentuating cholinergic ing that M35 is primarily interacting with GAL receptors<br>labeled by  $[^{125}I]pGAL$ .<br>The other possible explanation for the complete inhibition<br>of  $[^{125}I]pGAL$  or  $[^{125}I]GLT$  binding by M35 in the BF is the<br>fact that thes labeled by  $[1^{25}I]pGAL$ .<br>The other possible explanation for the complete inhibition<br>of  $[1^{25}I]pGAL$  or  $[1^{25}I]GLT$  binding by M35 in the BF is the<br>fact that these ligands bind irreversibly. To address this<br>issue, order The other possible explanation for the complete inhibition CHEN,<br>of  $[^{125}]$ JpGAL or  $[^{125}]$ GLT binding by M35 in the BF is the<br>fact that these ligands bind irreversibly. To address this<br>issue, order of addition of compe of  $[1^{25}I]pGAL$  or  $[1^{25}I]GLT$  binding by M35 in the BF is the fact that these ligands bind irreversibly. To address this considered issue, order of addition of competitor or radioligand was considered. Either M35 or ra fact that these ligands bind irreversibly. To address t<br>issue, order of addition of competitor or radioligand v<br>considered. Either M35 or radioligand was added directly<br>the BF homogenates and preincubated before the start issue, order of addition of competitor or radioligand was considered. Either M35 or radioligand was added directly to the BF homogenates and preincubated before the start of the binding assay. In these studies, complete i considered. Either M35 or radioligand was added directly to<br>the BF homogenates and preincubated before the start of the<br>binding assay. In these studies, complete inhibition of  $[^{125}]$ p-<br>GAL binding was still noted regardl binding assay. In these studies, complete inhibition of  $[^{125}]$ <br>GAL binding was still noted regardless of order of addition<br>Therefore, kinetics or other neuropeptide receptor interfection<br>ence does not appear to be the re GAL binding was still noted regardless of order of addition. DEECHER, D. C., PAYNE, G. T. AND SODERLUND, D. M.: Inhibition of [<sup>3</sup>H] batra-<br>Therefore, kinetics or other neuropeptide receptor interfer-<br>ence does not appear Therefore, kinetics or other neuropeptide receptor interference does not appear to be the reason for the complete inhibition of binding. It is possible that M35 is causing a conformational change of the receptor, inhibitin ence does not appear to be the reason for the complete inhibition of binding. It is possible that M35 is causing a conformational change of the receptor, inhibiting the binding of the pradioligands. Further studies are req tissue. ational change of the receptor, inhibiting the binding of the  $\frac{DE}{DE}$ <br>dioligands. Further studies are required to fully determine<br>e effect of M35 on the GAL receptor described in the human<br>sue.<br>In conclusion, we charact radioligands. Further studies are required to fully determine<br>the effect of M35 on the GAL receptor described in the human<br>tissue.<br>In conclusion, we characterized GAL receptors in human<br>BF and HYP using two radioligands,

the effect of M35 on the GAL receptor described in the human<br>tissue.<br>In conclusion, we characterized GAL receptors in human<br>BF and HYP using two radioligands,  $[^{125}]$ pGAL and<br> $[^{125}]$ GLT. No apparent age-related differenc In conclusion, we characterized GAL receptors in human<br> *BF* and HYP using two radioligands,  $[1^{25}I]pGAL$  and<br>  $[1^{25}I]GLT$ . No apparent age-related differences in GAL recep-<br> *vitro*, are high-affinity agonists and irre BF and HYP using two radioligands,  $[^{125}I]pGAL$  and  $[^{125}I]GLT$ . No apparent age-related differences in GAL receptor pharmacology were noted in BF. Both radioligands, in *vitro*, are high-affinity agonists and irreversi [<sup>125</sup>I]GLT. No apparent age-related differences in GAL receptor pharmacology were noted in BF. Both radioligands, in *vitro*, are high-affinity agonists and irreversibly bind to GAL receptors in the BF and HYP, which is i tor pharmacology were noted in BF. Both radioligands, in<br>
uitro, are high-affinity agonists and irreversibly bind to GAL<br>
receptors in the BF and HYP, which is in line with the theory<br>
of tight agonist binding. Our study s

*Vol. 275*<br>GAL receptor depending on the radioligand or brain region<br>selected. At the time of the present study,  $[^{125}I]hGAL$  was not *Vol. 275*<br>GAL receptor depending on the radioligand or brain region<br>selected. At the time of the present study,  $[^{125}I]hGAL$  was not<br>available, and further characterization must be done with  $V$ ol. 275<br>GAL receptor depending on the radioligand or brain region<br>selected. At the time of the present study,  $[$ <sup>125</sup>I]hGAL was not<br>available, and further characterization must be done with<br>the specific human peptide GAL receptor depending on the radioligand or brain region selected. At the time of the present study,  $[^{125}I]hGAL$  was not available, and further characterization must be done with the specific human peptide to compare an selected. At the time of the present study,  $[1^{25}]$ lhGAL was not available, and further characterization must be done with the specific human peptide to compare and contrast receptor available, and further characterization must be done with

available, and further characterization must be done with<br>the specific human peptide to compare and contrast receptor<br>differences between radioligands and regions tested.<br>Based on several reports, GAL may be a potential ph the specific human peptide to compare and contrast receptor<br>differences between radioligands and regions tested.<br>Based on several reports, GAL may be a potential pharma-<br>cological treatment strategy for BF dysfunction in A differences between radioligands and regions tested.<br>Based on several reports, GAL may be a potential phar<br>cological treatment strategy for BF dysfunction in AD. Tl<br>defining GAL receptor pharmacology in normal brain tis<br>wi Based on several reports, GAL may be a poten cological treatment strategy for BF dysfunction if defining GAL receptor pharmacology in normal will be useful when characterizing the receptor ogy of the human BF from patients

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