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Modulation of Conditioned Fear, Fear-Conditioned Analgesia, and Brain Regional C-Fos Expression Following Administration of Muscimol into the Rat Basolateral Amygdala

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Abstract: Evidence suggests that gamma-aminobutyric acid (GABA) signalling in the basolateral amygdala (BLA) is involved in pain, fear, and fear-conditioned analgesia (FCA). In this study, we investigated the effects of intra-BLA administration of the $GABA_A$ receptor agonist muscimol on the expression of conditioned-fear, formalin-evoked nociception, and fear-conditioned analgesia in rats, and the associated alterations in brain regional expression of the immediate early gene product and marker of neuronal activity, c-Fos. Formalin-evoked nociceptive behavior, conditioned-fear and fear-conditioned analgesia were apparent in animals receiving intra-BLA saline. Intra-BLA muscimol suppressed fear behavior and prevented fear-conditioned analgesia, but had no significant effect on the expression of formalin-evoked nociception. The suppression of fear behavior by intra-BLA muscimol was associated with increased c-Fos expression in the central nucleus of the amygdala (CeA) and throughout the periaqueductal grey (PAG). These intra-BLA muscimol-induced increases in c-Fos expression were abolished in rats receiving intraplantar formalin injection. These data suggest that alterations in neuronal activity in the CeA and PAG as a result of altered GABAergic signalling in the BLA may be involved in the behavioral expression of fear and associated analgesia. Furthermore, these alterations in neuronal activity are susceptible to modulation by formalin-evoked nociceptive input in a state-dependent manner.

***Perspective:** The expression of learned fear and associated analgesia are under the control of $GABA_A$ receptors in the basolateral amygdala, through a mechanism which may involve altered neuronal activity in key components of the descending inhibitory pain pathway. The results enhance our understanding of the neural mechanisms subserving fear-pain interactions.*

***Key words:** Amygdala, muscimol, $GABA_A$ receptor, fear, pain, analgesia, rat, c-Fos.*

Fear can elicit complex changes in neuronal processing in the descending inhibitory pain pathway. Brain regions critically involved in the descending inhibitory pain pathway include the basolateral amygdaloid complex (BLA), central nucleus of the amygdala (CeA), periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM), and neuronal activity in these regions also subserves expression of fear. A large proportion of patients suffering from persistent pain often report with comorbid anxiety disorders,[1] and evidence suggests altered pain processing in patients suffering from anxiety disorders such as posttraumatic stress disorder.[17] Thus, studies investigating neuronal mechanisms in brain regions commonly implicated in both pain and fear may provide useful information that could be exploited for therapeutic gain.

Fear-conditioned analgesia is a phenomenon whereby animals reexposed to a neutral context (eg, conditioning arena) which has been previously paired with an aversive

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stimulus (eg, footshock) display conditioned analgesia.[14,15,20,24] Information relating to context or aversion/nociception which is processed in brain regions including the thalamus and cortex converges at the level of the BLA and CeA.[46] During fear conditioning, it is postulated that the convergence of information is reinforced after each footshock. Subsequent reexposure to the context where the aversive events occurred results in hyperexcitability and increased plasticity of neurons in the BLA,[55] and these neurons further relay the information through GABAergic intercalated cells[57] to the CeA. The medial sector of the CeA is thought to be the main source of amygdalar outputs to the PAG and hypothalamic sites responsible for fear behavior.[4,12,31] Indeed, neuronal projections from the CeA to the PAG[52] are strongly involved in the endogenous aversive and analgesic systems. The activation of this pathway results in both the expression of fear-related behavior (eg, freezing and 22-kiloHertz ultrasonic vocalizations)[10,49] and robust analgesia as a consequence of activating the descending inhibitory pain pathway.[27,32,37,43]

GABAergic neurotransmission in the BLA, CeA, and PAG plays a key role in supraspinal modulation of pain,[13,50], fear,[5,9,51,55,58] and fear-conditioned analgesia.[19-21] A previous microdialysis study from our laboratory reported a significant suppression of GABA levels in the BLA of fear-conditioned rats compared with non-fear-conditioned controls, suggesting that reduced GABAergic signalling in the BLA may facilitate the expression of conditioned fear.[51] The present study tested the hypothesis that $GABA_A$ receptor activation in the BLA attenuates the expression of conditioned fear and fear-conditioned analgesia in rats. A pharmacological approach was adopted, employing microinjection of the $GABA_A$ receptor agonist muscimol bilaterally into the BLA. Furthermore, we investigated associated alterations in the expression of the immediate early gene product, c-Fos, as an index of altered neuronal activity in the CeA and PAG.

Methods

Animals

Male Lister-hooded rats (280–350 g; Charles River, Margate, Kent, UK) were used. Animals were housed 4 per cage before surgery and were maintained at a constant temperature (21 $\pm$ 2°C) under standard lighting conditions (12:12 hour light-dark, lights on from 0800 to 2000). Experiments were carried out during the light phase between 0800 and 1700. Food and water were available ad libitum. The experimental protocol was carried out following approval by the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under license from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609.

Cannulae Implantation

Stainless steel guide cannulae (Plastics One Inc., Roanoke, VA) were stereotaxically implanted 1 mm above the right and left basolateral amygdala (AP −.25 cm, ML $\pm$ .48 cm relative to bregma, DV −.71 cm from skull surface;[48] under isoflurane anaesthesia (2-3% in O_2; .5 L/minute). The cannulae were permanently fixed to the skull using stainless-steel screws and carboxylate cement. The duration of surgery from initial anaesthesia until recovery was 50 to 70 minutes. A stylet made from stainless steel tubing (Plastics One Inc.) was inserted into the guide cannula to prevent blockage by debris. 250 μL of the nonsteroidal anti-inflammatory agent, carprofen (.5% sc) (Rimadyl; Pfizer, Kent, UK), and 250 μL of the broad spectrum antibiotic, enrofloxacin (.5% sc) (Baytril; Bayer Ltd., Dublin, IE) were administered before the surgery to manage postoperative analgesia and to prevent infection respectively. Following cannulae implantation, the rats were singly housed and administered enrofloxacin (Baytril) for a further 3 days. At least 6 days postsurgery were allowed for recovery prior to experimentation. During this period, the rats were handled and their body weight and general health monitored on a daily basis.

Drug Preparation

The $GABA_A$ receptor agonist muscimol (Sigma-Aldrich, Dublin, IE) was freshly prepared on test days at a concentration of 1.0 mg/mL in sterile saline. 2.5% formalin (Sigma-Aldrich) solution was also freshly prepared in sterile saline on test days.

Experimental Procedures

The experimental procedure was essentially as described previously.[6,15,16,51,53,54] In brief, it consisted of 2 phases, conditioning and testing, occurring 24 hours apart. Subjects were randomly assigned to groups and the sequence of testing was randomized. On the conditioning day, rats were placed in a Perspex fear-conditioning/observation chamber (30 $\times$ 30 $\times$ 30 cm) and after 15 seconds they received the first of 10 footshocks (.4 mA, 1-second duration; LE85XCT Programmer and Scrambled Shock Generator; Linton Instrumentation, Norfolk, UK) spaced 60 seconds apart. Fifteen seconds after the last footshock, rats were returned to their home cage. Controls not receiving footshock were exposed to the chamber for an equivalent 9.5-minute period.

The test phase commenced 23.5 hours later when the animals were placed under brief isoflurane anaesthesia (3% in O_2; .5 L/minute). At this time, animals received intra-BLA microinjection of either muscimol (MUSC .5 μg/.5 μL) or sterile saline (VEH .5 μL) into the right and left BLA 60 to 90 seconds prior to formalin administration. Subjects received an intra-plantar injection of 50 μL formalin (Form 2.5% in .89% NaCl) or saline (Sal .89% NaCl) into the right hindpaw. The dose of muscimol (.5 μg/.5 μL/side) was chosen based on studies from the literature demonstrating that intracerebral administration of muscimol suppressed the expression of conditioned fear.[34,38] A full description of the injection procedure has been published previously.[54] This design resulted in 8 experimental groups as illustrated below.