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Additional evidence for the involvement of the basal ganglia in formalin-induced nociception: the role of the nucleus accumbens

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Abstract

Identification of the brain areas that contribute to pain is an essential undertaking towards understanding persistent pain. Areas of the basal ganglia have been proposed to play important roles in nociception as previous studies have determined the involvement of the substantia nigra pars compacta and the dorsolateral striatum in pain. The purpose of the present study was therefore to expand upon these findings by determining the involvement of other areas of the basal ganglia such as the nucleus accumbens shell and core in formalin-induced nociception. It was found that injection of a local anaesthetic (bupivacaine) into the nucleus accumbens shell had no effect on formalin-induced nociception. However, injection into the nucleus accumbens core enhanced formalin-induced nociception. These results implicate the nucleus accumbens in the processing of pain and provide additional evidence for the involvement of the basal ganglia and possibly dopamine in pain.

Theme: Sensory systems

Topic: Pain: pathways

Keywords: Nucleus accumbens; Nociception; Striatum; Substantia nigra; Formalin test; Basal ganglia; Dopamine

1. Introduction

The complex and multidimensional nature of pain requires integration of sensory-discriminative, motivational-affective, cognitive, and motoric components [20,21]. These components provide perceptual information regarding the location, magnitude, and spatiotemporal properties of a noxious stimulus as well as cognitive, learning and motivational responses which contribute to the organism's perception of, and response to, this stimulus. Considering the many factors that contribute to pain, a system is required that is capable of coordinating information relating to a noxious stimulus, identifying those stimuli to which an organism must attend to, and formulating the appropriate response.

The basal ganglia play an important role in sensorimotor integration as they receive somatosensory information and provide information to areas of the brain responsible for motor behaviours [15]. The incoming sensory information may be used to select or modify behaviours in response to environmental factors such as noxious stimuli, and therefore areas of the basal ganglia likely play important roles in the processing of pain. In our previous studies looking at the involvement of the basal ganglia in nociception we investigated the role of the substantia nigra and dorsolateral striatum in formalin-induced nociception.

The role of the substantia nigra in nociception has been determined by studies demonstrating that both the substantia nigra pars compacta (SNc) and pars reticulata (SNr) contain neurons that are responsive to noxious stimulation [5,6,16,17,24–27,29,30]. Furthermore, it has been proposed that some nociceptive nigral neurons can encode stimulus intensity and may therefore play a role in the sensory-discriminative dimension of pain [8]. Our studies of the substantia nigra demonstrated that injection of a local anaesthetic into the SNc, but not the SNr, disrupted formalin-induced nociception [18]. These findings implicated the involvement of not only the SNc but also other

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areas of the basal ganglia by way of such projections as the nigrostriatal pathway.

Studies have shown that a high proportion of striatal neurons are responsive to somatosensory stimulation and are activated differentially or exclusively by noxious stimuli thus implicating this brain area in pain [8]. In our study investigating the involvement of the dorsolateral striatum (DLST) and dopamine in the formalin model of persistent nociception we found that injection of D_2, but not D_1, agonists and antagonists into this region influenced formalin-induced nociception [19]. Based on these findings, we concluded that the DLST and dopamine play important roles in nociceptive processing [19].

Having determined that the DLST and SNc are involved in nociception, the present study was undertaken to investigate the involvement of other areas of the striatum in pain processing. The striatum consists of several divisions which interact by way of a complex projection system. In addition to the DLST, the ventral striatum consists of various nuclei including the ventral portions of the caudate nucleus and putamen, deep layers of the olfactory tubercule and the nucleus accumbens [7]. Of particular interest in relation to pain is the nucleus accumbens (NuAc).

The NuAc is divided into the shell and core. The shell projects to the ventromedial ventral pallidum which projects to the ventral tegmental area (VTA) whereas the core projects directly to the lateral VTA and the SNc [10,22]. As the NuAc interacts with areas of the basal ganglia implicated in pain processing, it is likely that the NuAc is also involved in nociception. Previous studies using the formalin model have proposed that the NuAc is involved in pain as it was found that morphine and amphetamine-induced analgesia involved increased dopamine levels in the NuAc [13]. Furthermore, fMRI studies have shown that the NuAc is activated during pain relieving sensations of acupuncture [31].

Although some studies have shown that the NuAc may be involved in pain, it remains to be determined if these effects are mediated by the NuAc shell or core. The purpose of the present study was therefore to determine the involvement of the NuAc shell and core in formalin-induced nociception.

2. Methods

2.1. Subjects and housing

Male Long-Evans hooded rats weighing 300–350 g at the time of surgery served as subjects. Animals were housed individually with food and water available ad lib and maintained under a 12:12 illumination cycle (light onset 07:00 h).

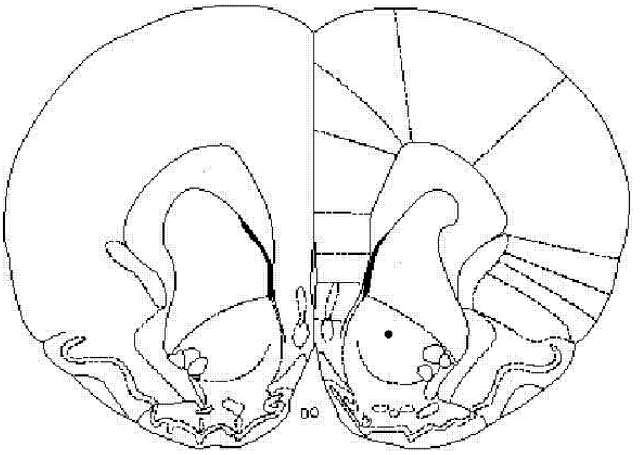

Fig. 1. Location of cannula tips for NuAc shell site.

2.2. Surgery

Under sodium pentobarbital anaesthesia (60 mg/kg, i.p.), rats were implanted unilaterally with a 23-guage cannula (Plastics One, Roanoke, VA, USA) aimed at the NuAc shell (AP +2.7, ML +1.3, DV −4.0; see Fig. 1) [23] or core (AP +1.78, ML +1.5, DV −4.0; see Fig. 2) [23]. Each cannula was anchored into place with dental cement poured around the cannula and two jeweler's screws, which were placed in the skull. A dummy cannula extending 0.5 mm beyond the tip of the outer cannula was inserted and remained in place until the time of formalin testing.

2.3. Formalin test and micro-injections

After a 7–10 day recovery period, animals were run in the formalin test. The formalin test was performed as previously described [12]. Animals were observed in a

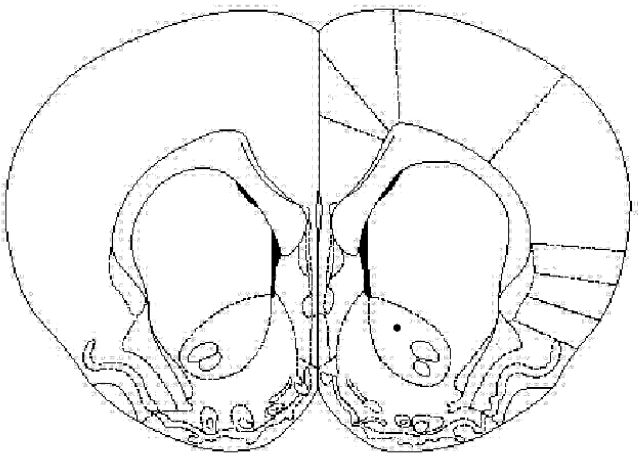

Fig. 2. Location of cannula tips for NuAc core site.

clear plastic Plexiglass box (32×32×16 cm), with a mirror positioned at a 45° angle below the floor thus allowing for unobstructed observation of the animal's paw. Following a 5-min habituation period, animals were removed from the box and the dummy cannula was removed from the outer cannula.

An inner cannula extending 2.6 mm beyond the end of the outer cannula was then inserted and 0.5 μl of either 0.25% bupivacaine or saline was injected over a 1-min period. Following infusion of the fluid, the inner cannula remained in place for an additional minute in order to prevent the reflux of fluids. Five minutes later, animals were injected with 50 μl of 1% formalin subcutaneously into the plantar surface of the hind-paw contralateral to the implanted cannula. The amount of time the injected paw was elevated was recorded in 5-min intervals during the 70-min period following formalin injection.

2.4. Open-field test and micro-injections

As the NuAc is involved in motor behaviours, the open field test was used to determine whether micro-injection of bupivacaine into the NuAc affected the animals' motor behaviour (i.e. their ability to elevate their paw following the formalin injection). As a significant difference was found between the core, but not the shell, group we investigated the impact of injections into the NuAc core on motor behaviour.

Animals were implanted with a cannula aimed at the NuAc core as outlined in the previous experiments. Following a 7–10 day recovery period, animals were placed in an open field which consisted of an open area (4 ft.× 8 ft.) with a grid floor (12 in.×12 in. squares) [19]. Animals were injected with 1.0 μl of saline or bupivacaine over a 2-min period. Animals were then returned to their home cages for 30 min, which allowed for observations to be made during the time in which the greatest effects were seen in the previous experiments. Animals were then placed in the open field and observed for a 20-min period. Motor activity was measured as the number of lines crossed and the amount of time the animal spent moving.

2.5. Histology

Once formalin or open field testing was complete, animals were overdosed with sodium pentothal (25 mg/ml, i.p.) and perfused transcardially with physiological saline followed by 10% formalin. Brains were then removed and fixed in a 20% sucrose formalin solution for 5 days prior to being cut with a microtome (brains were cut at a cryostat temperature of −20 °C). Verification of cannula tip placement was made from 40 μm coronal sections stained in cresyl violet.

3. Results

Of the 38 animals implanted, 14 rats had cannula tip placements in the NuAc shell (saline, $n=8$; bupivacaine, $n=6$) and 15 rats had cannula tips in the NuAc core (saline, $n=8$; bupivacaine, $n=7$). Independent t-tests were conducted for the NuAc shell and core data for the amount of time animals elevated their paws in the early (0–10 min) and late (15–70 min) phases of the formalin test.

No significant differences were found between the shell saline and bupivacaine groups. However, a significant group effect was found between the core saline and bupivacaine groups for the amount of time animals elevated their paw in the late phase ($t(13) = -2.22$, $P = 0.045$). No significant difference was found between the core saline and bupivacaine groups in the early phase of the formalin test (see Fig. 3).

In the open field test, no significant difference was found between groups (saline, $n=7$; bupivacaine, $n=6$). These results demonstrate that animals were not affected in their ability to make the motor responses required in the formalin test (i.e. paw elevation).

4. Discussion

In this study, we demonstrated that micro-injection of a local anaesthetic (bupivacaine) into the NuAc influences responding in the formalin test. These results are consistent with previous studies indicating the involvement of the NuAc in pain [1–3,14]. Since micro-injection of bupivacaine did not influence motor behaviour of the animals, it is unlikely that the obtained effects in the formalin test are attributable to altered motor behaviours.

It is well established that the formalin test consists of two phases [12,28]. In the present study we have demonstrated that injection of bupivacaine into the NuAc core, but not the shell, enhanced formalin-induced nociception in the late phase of the formalin test. These findings are consistent with previous studies which have shown that the early and late phases of the formalin test involve different mechanisms [9,11,12] and specifically that differences exist within the NuAc and its pathways in relation to responses in the formalin test [1].

The differences shown between the NuAc core and shell's involvement in modulating persistent nociception is interesting as these structures are anatomically distinct but interact via common pathways. The anatomical distinction between the NuAc core and shell relates to their location within the ventral striatum. The NuAc core is located in the dorsolateral portion of the ventral striatum and the shell is in the medioventral portion of the ventral striatum [32]. Although anatomically distinct, the NuAc core and shell interact as the NuAc shell modulates the core through the VTA by way of dopaminergic and GABA pathways [22].

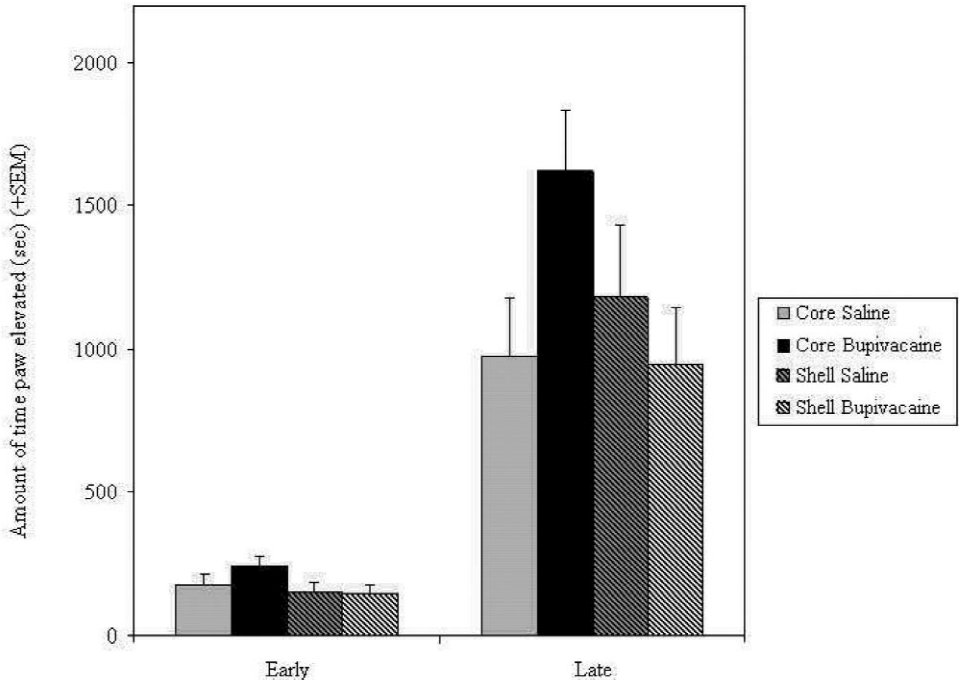

Fig. 3. Mean time paw elevated during the early and late phases of the formalin test.

The VTA gives rise to ascending midbrain dopaminergic neurons which then project to the NuAc [3]. This pathway has been shown to play a role in pain. For example, in the study by Altier and Stewart [2], dopamine antagonists injected into the NuAc blocked the analgesic effects of intra-accumbens or intra-VTA SP, morphine and amphetamine. In addition to providing additional evidence for the involvement of the NuAc and dopamine receptors in the inhibition of pain, Alteir and Stewart state that the NuAc is the neuroanatomical site where these receptors mediate their anti-nociceptive response [2]. Additional evidence for the role of the NuAc and its related dopaminergic pathways in nociception comes from a study by Deutch and Cameron [10], which found that dopaminergic responses increase in the core during sustained responses whereas they increase in the shell at the onset of a stimulus. These results indicate that the NuAc core is involved in persistent aspects of behaviours such as those of ongoing pain whereas the shell is involved in the onset of a painful stimulus. The findings of Deutch and Cameron [10] provide additional support for the present findings as we found a difference between core groups in the late, but not the early, phase of the formalin test. As the NuAc core has a higher density of D_2 receptors than the shell [4], dopaminergic effects may be involved with the results of the present study as disruption of the NuAc core would have disrupted functioning of this brain area including that mediated by dopamine. This possibility is consistent with previous studies demonstrating the involvement of the basal ganglia and D_2 receptors in nociception [19].

Although no significant differences were found between NuAc shell groups in the present study, as the shell interacts with the core by way of the VTA it is possible that the results obtained in the shell involved the core as well. As proposed by Altier and Stewart [2], tonic pain is inhibited in part by enhanced dopamine released from terminals of mesolimbic neurons and, furthermore, the pain-suppressing system involving the activation of these neurons is naturally triggered by exposure to stress and/or pain [2]. Therefore, an injection of bupivacaine into the NuAc core would disrupt the engagement of this anti-nociceptive dopaminergic system thus enhancing nociception. This is consistent with the findings of the present study as injection of bupivacaine into the NuAc core enhanced formalin-induced nociception. In contrast, injection into the NuAc shell would not have disrupted this effect, as the VTA would activate the dopaminergic system in response to the pain of formalin injection independent of the NuAc shell thus preventing an enhancement of formalin-induced nociception. This possibility is consistent with the present findings as injection of bupivacaine into the NuAc shell did not significantly alter animals' responses in the formalin test.

In summary, the present results confirm the involvement of the NuAc in nociception and, further, implicate the involvement of the NuAc core, but not the NuAc shell, as playing a direct role in the modulation of persistent nociception. Additionally, by way of the dopaminergic pathways between the areas of the NuAc and other areas of the brain, these results provide additional evidence for the involvement of dopamine in nociception. Further studies are needed to elucidate the specific roles of the different

areas of the NuAc and the related dopamine systems, as well as other neurochemical modulators within these brain structures in modulating pain. We are presently undertaking experiments to further investigate the role of dopamine within the NuAc in persistent nociception. Such studies will facilitate our understanding of the mechanisms that contribute to nociception and the multidimensional experience of pain.

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